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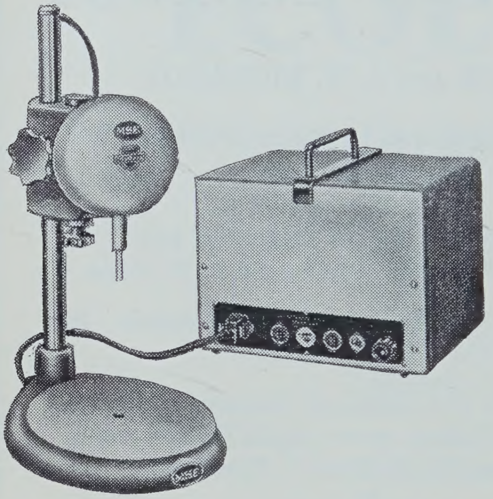

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Reorganization of Root Apices after Irradiation

BY

F. A. L. CLOWES

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ABSTRACT

Roots were irradiated with X-rays and then fed with (8–C^{14}) adenine at various intervals afterwards to see the effect of the radiation on the sites of DNA synthesis and hence on the behaviour of the meristem. The dividing cells of the meristem may be so badly damaged by the X-rays that they stop synthesizing DNA and dividing. When this happens the root may continue to grow by forming a new meristem. This often arises in the quiescent centre whose cells normally do not synthesize DNA or divide. These therefore form a reservoir of cells which are less vulnerable because of their quiescence, but are able to restart DNA synthesis and division when the normally meristematic cells stop. Because of this reorganization of the apex, it is not legitimate to argue about the behaviour of normal root meristems from chimeras induced by irradiation.

INTRODUCTION

IN root apices the cells are arranged in patterns which reflect their origin. It is possible to interpret these patterns to show how a root is constructed in its ontogeny. The most important of the limitations of such analyses in Angiosperms is the identification of initials, the cells which initiate new tissues. In many Pteridophytes it is agreed that there is a single initial, 'the apical cell', which initiates all the tissues. In Angiosperms it is often thought that there are several initials constituting a promeristem, but the size and site of the promeristem is controversial.

By following the patterns of cells a minimal constructional centre can be derived (Clowes, 1954). In the past most anatomists have assumed that this was identical with the promeristem. Guttenberg and his associates believe this assumption to be correct and reduce the size of the promeristem to a single 'central cell' or to a few initial cells (Guttenberg, 1947; Schade and Guttenberg 1951). This view can be interpreted in two ways. Either the 'central cell' can function, like the apical cell of a Pteridophyte, throughout the growth of the root, or it can function only in the embryo or primordium and give rise later to a multicellular promeristem in the root. Guttenberg originally seemed to favour the former interpretation, but recently has stressed the latter one (Guttenberg, Heydel, and Pankow, 1954 *a*, *b*; Guttenberg, Burmeister, and Brossel, 1955).

In apices with clearly defined histogens such as grass roots have, analysis of the patterns of cells has led to a different view. In this the promeristem is considered to consist of many cells situated on the surface of a quiescent centre whose cells divide rarely or never in normal roots. Although such an

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anatomical analysis cannot be done satisfactorily on other kinds of roots the theory is believed to be generally applicable to Angiosperm roots. The theory is supported by determinations of relative rates of nucleic acid and protein synthesis (Clowes, 1956 *a*, *b*, 1958 *a*, *b*) and by surgical experiments (Clowes, 1953, 1954). The promeristem in this theory consists of many more cells than the minimal constructional centre. Going back to the embryo, it is possible to derive the whole of the root from a single cell, but it is questionable whether a single totipotent cell ever exists when the root meristem is a recognizable structure (Steffen, 1952). However, it can be shown that the quiescent centre is at first absent in embryos and lateral primordia and does not appear until the meristem is well developed (Clowes, 1958 *a*).

The one striking piece of evidence that has been put forward for believing in a very small number of initials is provided by Brumfield's production of cytological chimeras in roots. Brumfield (1943) irradiated with X-rays roots of *Vicia faba* and *Crepis capillaris* when 30 mm. long and 2 mm. long respectively. The dose was 1,000 r. for *Crepis* and 300 r. for *Vicia*. The roots were then allowed to grow until they were 40–50 mm. long in *Crepis* and 100–120 mm. long in *Vicia*. The apices were sectioned and Brumfield observed several kinds of unequal reciprocal translocations in the chromosomes in the meristems. The aberrations occurred in several cells assumed to be derived from a single mutant cell and all of one kind of aberrant cell occurred in a sector of about 120° extending from the stele to the cap. The formation of these sectorial chimeras led Brumfield to postulate the existence of three initials for each root, each initial contributing a sector of each of the tissues. Brumfield's conclusion is therefore at variance with both the histogen theory and any theory which proposes a large promeristem.

Rickard (1952) has repeated Brumfield's experiment on *Crepis*, but obtained only a single chimerical root. This chimera was mericlinal and not truly sectorial as there was no evidence that aberrant cells of one type occurred in more than one histogen. Rickard concluded that the initials of *Crepis* existed in three tiers each of three cells. He therefore supports the histogen theory, but denies the hypothesis of a large promeristem.

The valuable results obtained by Satina and Blakeslee, Dermen, Thielke, and others (reviewed by Clowes, 1957) on natural and artificial chimeras in shoots led some people to expect that chimeras in roots would help in understanding the mechanics of root meristems. But the results have been disappointing. It has never been shown that roots could maintain stable chimeras and, from the behaviour of roots treated with colchicine, it seems that chimerical roots are unstable (Levan, 1938; Lewis, 1951). There is no mechanism such as the exclusively anticlinal divisions of the tunica layers of shoot apices which will maintain germ layers against mixing of cells in root apices. Therefore root meristems with aberrant nuclei need not maintain their normal behaviour.

Brumfield's conclusions about the number of initials in normal roots must be rejected for several reasons summarized by Clowes (1959). But it is also necessary to show how X-irradiation can produce sectorial chimeras, even unstable ones. The work reported here is an investigation of how a meristem changes its organization after its cells have been damaged by X-rays. It will be seen that the reorganization could lead to sectoring. Some of the histological changes which take place after irradiation have been described by Gray and Scholes (1951), but their conclusions were based on the classical view of meristem behaviour and would probably be modified if they accepted a quiescent centre.

TECHNIQUE

Several species of plants were used in these experiments, but *Zea mais* provides the most instructive material because the position of the quiescent centre can be determined more certainly than in other kinds of roots. *Vicia faba* will also be described because it was used by Brumfield and by Gray and Scholes. Young plants with primary roots 1–8 cm. long were irradiated with X-rays at 120 r./min. for 3 or 10 minutes and then replanted in moist sphagnum. *Vicia* roots are more sensitive to X-rays than *Zea* roots. The roots were examined 1–13 days after irradiation. The examination consisted of growing the roots in aerated solutions of adenine, labelled at position 8 with C^{14}, for 24 hours, fixing the apices in acetic-alcohol, sectioning at $6\,\mu$, hydrolysing in HCl to remove ribose nucleic acid, and preparing autoradiographs of the sections on stripping film emulsion. The adenine is taken up by the nuclei and incorporated into desoxyribose nucleic acid in those cells which are about to divide or become polyploid. Cells which are not about to divide or otherwise double their DNA content do not incorporate adenine under these conditions and therefore produce no autoradiograph over their nuclei.

OBSERVATIONS

In *Zea*, 360 r. produced no anatomical variations in the root meristem. There is a clear quiescent centre with no nuclear autoradiograph just as there is in normal roots. After irradiations of 1,200 r. some, but not all, of the root meristems became disorganized. When fed with labelled adenine 2 days after irradiation the roots showed a clear quiescent centre, and the only anatomical sign of abnormality was the absence of nuclear autoradiograph from some small areas of the cortex where it would have existed in normal roots. When a longer interval exists between irradiation and feeding with adenine the absence of nuclear autoradiograph becomes more marked especially in the cortex of those roots where it occurs.

When the interval between irradiation and feeding is 4 days the quiescent centre is smaller than usual in some meristems. The cells near the surface of the quiescent centre have restarted to synthesize DNA. When the interval is 6 or 8 days the quiescent centre is often completely absent and the cells in the position of the quiescent centre may be almost the only ones with nuclear autoradiograph. When this occurs the boundary between the cap and the rest of apex, which is normally very distinct in *Zea*, may disappear and the meristem is obviously organized quite differently from that of a normal root. Some roots become moribund and die before this stage is reached.

In *Vicia* irradiated with 1,200 r. the roots grow for about 3 days and then stop. Cell division occurs, but becomes increasingly rare and micronuclei are formed in many of the cells. By the 7th day no meristem exists at all.

After 360 r. most meristems also become disorganized, but the roots usually survive. Most of the cells of the main apical meristem commonly stop dividing and a smaller meristem is formed either in a lateral position near the apex in a group of meristematic cells of the outer stele and inner cortex, or in the quiescent centre. In a few roots two or three new meristems appear and these may produce a branched root. More commonly a single root, continuous with the original root, is produced. After the new meristem has grown the whole root appears to be a single structure as if the old root had continued growth from its original apical meristem. This happens whether the new meristem is derived from the quiescent centre or from a lateral meristem.

DISCUSSION

X-rays can affect meristems in several ways. They can lower the rate of DNA synthesis and thus cause a delay in mitosis by prolonging the presynthesis stage of interphase. They can also delay the entry into mitosis after DNA synthesis has begun (Howard and Pelc, 1953). They can break chromosomes, and the aberrant nuclei produced may be so unbalanced after the first mitoses that division may fail to occur again. Judged at the second or subsequent mitoses after irradiation, cells are more susceptible to breakage during mitosis and at the end of interphase than during the rest of interphase, and actively growing and dividing cells are more susceptible than others (Darlington, 1947; Davidson, 1958). There are also likely to be other disturbances in the cells due to ionization and these may affect the rate of division, and some may spread and behave as hormones.

All these injuries can combine to lower the growth-rate of a root by lowering the number of meristematic cells in the apex and slowing the mitotic cycle. Gray and Scholes (1951), for instance, calculated that X-radiation of 140 r. slows the mitotic cycle to three and a half times and the duration of mitosis to four and a half times the normal in *Vicia* roots after 4 days. It also reduces the number of dividing cells in the apex to nearly a half. These effects reduce the growth rate to one-fifth of the normal.

The present observations show that the quiescent centre, which consists of about 500 cells in *Zea* and 1,100 cells in *Vicia*, behaves as a reservoir of cells which will become meristematic when the surrounding cells have lost their ability to divide at a normal rate. Presumably some of the cells of the quiescent centre have been affected less by the radiation than have the cells of the rest of the meristem though their chromosomes may have been broken in interphase. Some of the formerly quiescent cells are therefore able to start synthesizing DNA and dividing to form a new apex when the other cells have lost this ability. The maintenance of such a reserve of cells relatively immune to injury may be beneficial to the plant.

It is also clear that conclusions about the behaviour of normal meristems may not legitimately be drawn from irradiated roots. The apical meristem may not have a continuous existence after irradiation as it does in normal roots. If the root survives the experiment and goes on growing it may do so only by producing a new apical meristem from the cells which are less badly damaged. The new apical meristem is derived from a number of cells which is small at the time of irradiation. If any of these cells has a nuclear aberration which is not lethal it may be detected in the new meristem. If the number of cells initiating the meristem is small then a particular aberration may be found in a large proportion of the cells of the new meristem. It may be that two or three quiescent cells will start dividing when their neighbours are unable to do so. Their progeny would form the new meristem and, if one of the cells was aberrant, the new root would be chimerical and probably sectorial, for the early part of its life at any rate. The stability of the chimera would depend upon whether the aberrant cells divide at the same rates as normal cells or not.

Davidson (1959) described the presence of aberrant cells in the branches produced by primary roots of *Vicia* which had been irradiated with X-rays three to four weeks beforehand. In one lateral meristem he found six different kinds of cells existed within a small region of the pericycle at the time of initiation of the lateral primordium. The impression given by this observation is very different from Brumfield's interpretation of his sectorial chimeras. It can be seen from the present work that the fact that Brumfield found no more than three kinds of cells does not mean that only three cells initiate the meristem during normal growth.

ACKNOWLEDGEMENTS

I am indebted to Dr. D. Davidson for discussions on this work and to the Royal Society for financial assistance.

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Floral Abnormalities in Broccoli[1]

BY

D. R. SAMPSON AND MARY MacARTHUR

With two Plates

ABSTRACT

Floral abnormalities, which apparently arise spontaneously in green sprouting broccoli, are described. Several types of abnormalities may develop on a raceme. Conspicuous among these are flowers with united sepals which bear ovules ventrally on placentae along the lines of sepal union, and pads of stigmatic tissue at the apices of the sepals. Other abnormalities include open, boat-shaped gynoecia, and irregular terminal structures suggestive of laterally united gynoecia. Developmental aspects of the abnormalities are discussed and their morphogenetic implications are noted.

INTRODUCTION

TERATOLOGICAL structures are caused by disturbances in normal developmental processes. Analysis of abnormalities, which nevertheless have points of similarity with normal structures, may provide some insight into the normal processes of development. Certain floral abnormalities in 'Italian Green Sprouting' broccoli (*Brassica oleracea* var. *italica*) are considered with this in mind.

Detjen and McCue (1933) reported abnormalities in cabbage (*B. oleracea* var. *capitata*) similar to those in broccoli, and we have seen these abnormalities on several plants of the cabbage variety 'Canadian Acre'. In addition, Haskell (1954) illustrated the same types of abnormalities in flowers of kohrabi (*B. oleracea* var. *gongyloides*) and offered the hypothesis that the abnormalities were reversions to primitive structures brought about by selection for tricotyly. Evidently the abnormalities are neither rare nor restricted to broccoli.

OCCURRENCE AND DESCRIPTION

The broccoli floral abnormalities were first observed late in the autumn of 1955 on plants growing in a cool greenhouse. Not all plants appeared to be affected, nor did abnormalities invariably develop on all racemes of an affected plant. When occurring, the abnormalities were terminal. Affected plants continued to produce abnormalities until May 1956, when most plants were discarded. However, one plant which was propagated by cuttings produced abnormalities until the time of writing (October 1957). Abnormalities were also observed on field-grown broccoli plants during the summers of 1956 and 1957, but these will be discussed later. The following descriptions are based on the 1955 greenhouse material.

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[1] Contribution No. 941 from the Horticulture Division, Central Experimental Farm, Canada Department of Agriculture, Ottawa, Canada.

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The 1955 greenhouse plants were used for pollination studies. Therefore each inflorescence was thinned to about six racemes to ensure flowering over a long period of time. This treatment is mentioned because, when all racemes are allowed to develop simultaneously, the length of an individual raceme is limited by abortion of all its terminal buds. Consequently, terminal abnormalities are more likely to be produced on thinned inflorescences.

From the base of an aberrant raceme normal flowers extended almost to the tip, but the distal five or six flowers had marginally united sepals. The extent of sepal connation varied from flower to flower, but usually the lowest abnormal flower had two or three sepals united, while the uppermost had all four united. Union of four sepals resulted in a tubular calyx, open at the apex. Partial union along a margin also occurred but this followed no definite pattern.

Ventrally, a cord-like strip of placental tissue was borne along each line of sepal union. A flower with four united sepals had four such placentae on which ovules, generally paired and normally oriented, were borne to the right and left (Fig. 1, Pl. 1). The free tips of the united sepals and even the margins where the union was partial bore pads of epidermal cells similar to normal stigmatic papillae (Fig. 5, Pl. 2). Dorsally, below this stigmatic tissue, arched lines similar to those that demarcate the distal margins of valves on normal gynoecia were sometimes evident (Fig. 3, Pl. 2). Flowers with united sepals usually had normal petals, stamens, and gynoecium; sometimes the usual complement of petals and stamens was present but, commonly, one or two petals or stamens were missing. An extreme flower had no petals and three stamens. Occasionally a stamen was united with the gynoecium, or a petal was joined to the calyx tube. When all four sepals were united, few of the petals and stamens emerged from the calyx tube (Fig. 2, Pl. 1) or, when only three sepals were united, the parts emerged from the open side of the calyx (Fig. 6, Pl. 2). The normal stigma usually emerged from the open mouth of the calyx tube (Fig. 5, Pl. 2) permitting pollination and, thereafter, silique development. United sepals usually remained attached to the flower long after the distinct sepals of normal flowers had fallen.

The six or so flowers with united sepals on an aberrant raceme were arranged according to the normal phyllotactic pattern. Distally, this orderly repetitive pattern of flower bud development was halted with the production of an irregular, terminal structure, which was often three times as long as a normal flower bud (Fig. 2, Pl. 2). Externally these structures bore valve demarcation lines and also stigmatic tissue around an apical opening. Internally the several longitudinal compartments contained ovules attached irregularly along, and occasionally between, the lines of union. Frequently, one or more compartments of such a structure were swollen. Dissection of the swellings invariably revealed a crumpled, imprisoned flower bud with united sepals. The compound nature of the terminal structures together with their possession of ovules, stigmatic cells, and valve lines suggests that they were composed of laterally united gynoecia. These 'fused gynoecia' were often immediately distal to the series of flowers with united sepals. Sometimes the lower part of the pedicel of a flower with united sepals was united with a 'fused gynoecia'. Occasionally a flower with united sepals located near a 'fused gynoecia' bore club-shaped structures (Fig. 6, Pl. 2), the apices of which were covered with cells resembling stigmatic papillae.

Frequently, although not invariably, the terminal 'fused gynoecia', and even the pedicels of subjacent flowers with united sepals, were subtended by bract-like, boat-shaped, 'open gynoecia' (Fig. 4, Pl. 2). These may be homologous with either sepals or single carpels. The 'open gynoecia' bore naked ovules attached ventrally both along the lateral margins and also some distance from the margins. Some of the ovules arising away from the margins were apparently normal; some, however, were reduced to elongated clubs, and others were represented only by funiculi. Marginally attached ovules were often grouped. Occasionally the funiculi of such ovules were digitately branched, and each division bore an apparently normal or partially developed ovule. There were no conspicuous cord-like placental strands on these 'open gynoecia'. The apices of the 'open gynoecia' bore thick inrolled pads of papillate stigmatic cells. A pad of stigmatic cells was once observed ventrally, at the base of an 'open gynoecium'.

One further floral abnormality in the broccoli plants deserves mention, although it was observed only once and so does not appear to be related in etiology to those described in the preceding. Naked ovules were observed on the external wall of an otherwise normal gynoecium. This gynoecium was not open along any carpel margin. The ovules were small, numerous, and extended in an oval band from the mid-region of one side of the ovary to well below its insertion. The band contained both orthotropous and campylotropous ovules. Crowding may have been responsible for the orientation of the former. Internally this ovary bore normal, ascending, campylotropous ovules. Sepals, petals, and stamens were normal.

Cause of the Abnormalities

We are unable to establish the cause of these floral abnormalities in broccoli. However, the evidence for and against several causal agents may be considered. Frost, mechanical injury, and artificial ionizing radiations were absent. Similarly, soil mineral deficiencies probably were not influential because none of the field-grown plants showed deficiency symptoms, and some of the greenhouse-grown plants which had abnormalities were raised by sand culture on Hewitt's (1952, p. 189) standard complete nutrient solution to which sodium nitrate had been added. Temperature was probably not a factor because abnormalities developed over the range 50° to 90° F.

Certain chemicals are known to incite abnormal growth. No record was kept of the agricultural chemicals used on the 1955 greenhouse plants but these records are available for the broccoli populations grown in the field in 1956 and 1957. In both years Metaldehyde slug poison ($(C_2H_4O)x$) was scattered near the young plants, and the plots were sprayed regularly with DDT and the fungicide Manzate (Manganous ethylene bisdithiocarbamate). The broccoli were not treated with herbicides.

In 1956, 120 plants were grown in the field and 118 developed floral abnormalities. Seedlings of all these plants had only two cotyledons so that the association between tricotyly and abnormalities noted by Haskell (1954) in kohlrabi was absent in our material.

Because the abnormalities may be genetically determined, the parentage of the 120 plants which were grown in 1956 is of interest. Sixty-nine plants, 68 of which developed abnormalities, belonged to a first inbred generation which was obtained by selfing a plant that developed abnormalities in the greenhouse in 1955. Forty-eight plants, 47 of which developed abnormalities, resulted from a cross between the original parent of the first inbred generation and an abnormal plant of the inbred generation. The inbred parent of this cross was also selfed, resulting in three plants, all with abnormalities. Thus the 120 plants were descendants of a single plant.

In 1957, only 15 of the 119 field-grown broccoli plants developed floral abnormalities. Genetically, none of the 1957 material was closely related to the plants on which abnormalities were found in 1955 and 1956. Table 1 lists the varieties grown in 1957 and shows the incidence of abnormalities in each variety. Not only is the frequency of abnormalities much lower than in 1956, but variety differences are evident. The two varieties, Green Mountain and De Cicco, are strictly comparable because they grew side by side in adjacent rows and flowered at the same time, yet one had a much higher incidence of abnormal plants than the other. This difference is statistically significant ($\chi^2 = 8{\cdot}8268, P < 0{\cdot}01$) and suggests that the abnormalities have a genetic basis.

TABLE 1

Numbers of Plants With and Without Floral Abnormalities in the Broccoli Varieties which were Field-grown in 1957

Variety	With abnormalities	Without abnormalities
Breeding Lines	2	24
Waltham No. 29	1	17
Early Purple Head	0	19
Green Sprouting	0	16
Green Mountain	10	9
De Cicco	2	19

DISCUSSION

A conspicuous feature of the broccoli floral abnormalities is the union of plant parts which are normally free. Sepal union is the simplest expression of this tendency and the first to appear on an abnormal raceme. In the development of a normal *Brassica* flower primordium, sepal primordia arise first and form a protective envelope within which stamen, carpel, and finally petal primordia arise (Thompson, 1933). The time difference in flower-part initiation could be a factor in explaining why stamens and petals are usually free in flowers with united sepals.

The terminal, irregular structures, which resemble laterally united gynoecia, represent maximum abnormal union. Their complexity suggests that several flower primordia contribute to their formation. Normally, *Brassica* flower primordia arise as spirally arranged protuberances on a raceme apex (Thompson, 1933). With increasing physiological disturbance as an abnormal raceme develops, it is possible that the tendency toward sepal union involves sepal primordia from separate but immediately adjacent flower primordia. If the terminal structures are formed in this way, their external walls would be homologous with sepals rather than with gynoecia. Further, it is possible that the laterally united flower primordia elongate faster than the raceme and finally entrap the raceme apex with its later formed flower buds.

The most interesting feature of the united sepals is that they regularly bear placentae, ovules, stigmatic papillae, and sometimes valve demarcation lines. These are gynoecial characters and, if we accept the hypothesis that the crucifer gynoecium is composed of united carpels, marginal union also may be considered a gynoecial character. The abnormal occurrence of these gynoecial characters shows hitherto unsuspected potentialities of sepal tissue. Further, the presence of the five characters on sepals demonstrates that the processes responsible for their normal development are independent of and separable from other attributes of the normal gynoecium, i.e. central location on the floral primordium, specialized vascularization, bi-lateral symmetry, and the development of a completely closed structure.

The fact that five different gynoecial characters regularly develop on sepals raises the question whether the several morphogenetic processes which result in the five characters are developmentally interconnected. Such an interconnexion of diverse morphogenetic processes is one possible mechanism by which the harmonious development of the several parts of an organism is achieved.

SUMMARY

1. The tendency toward abnormal union of parts in a broccoli raceme involves sepal primordia and possibly immediately adjacent flower primordia but not usually stamen or petal primordia.

2. United sepals regularly have placentae, ovules, stigmatic tissue, and 'valve lines'.

3. Development of five gynoecial characters on sepals shows that the normal development of these characters is independent of and separable from other events normally associated with gynoecial morphogenesis.

4. The presence of five different gynoecial characters on sepals suggests that the morphogenetic processes responsible for the five characters are developmentally interconnected.

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EXPLANATION OF PLATES 1 AND 2

PLATE 1

Fig. 1. A calyx with united sepals cut open to expose the ovules borne on a cord-like placenta along a line of sepal union. ×27·6.

Fig. 2. Terminal structure resembling laterally united gynoecia. The vesicle to the right was opened to expose the imprisoned flower bud. The flower to the left has united sepals, and arises below the terminal structure. ×6·7.

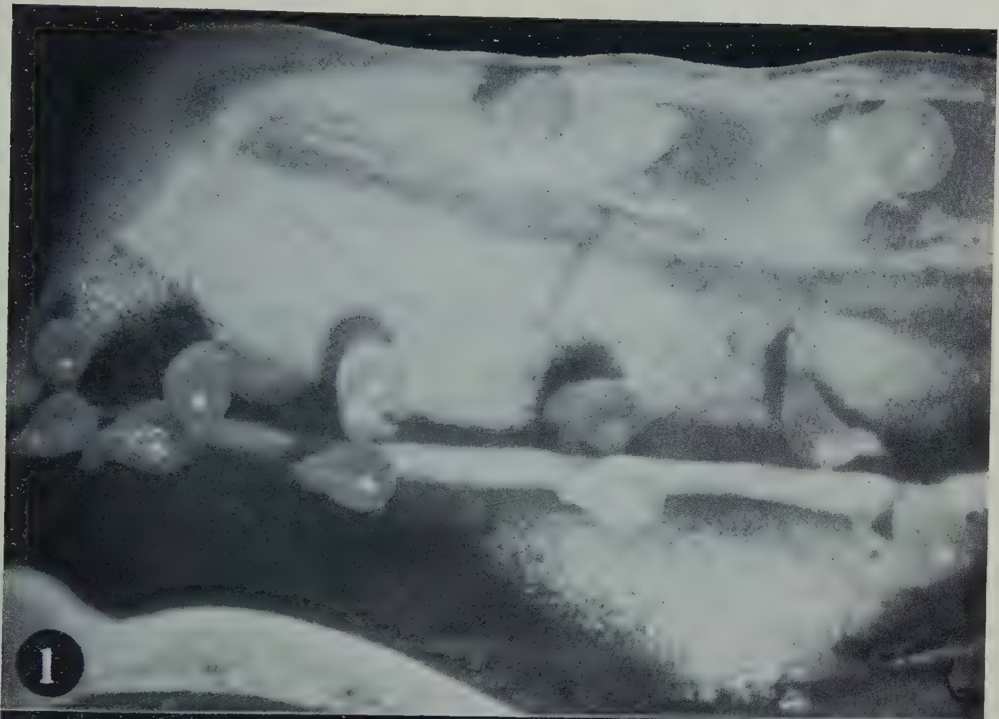
PLATE 2

Fig. 3. A flower bud with united sepals showing distinct lines of 'valve' demarcation near the apex. ×3·6.

Fig. 4. An open bract-like gynoecium with lateral ovules and an apical (left) pad of stigmatic papillae. ×12·5.

Fig. 5. The apex of a flower bud with united sepals. A normal stigma emerges from the opening which is surrounded by pads of stigmatic papillae. ×9·2.

Fig. 6. A flower with three united sepals; stamens and a petal emerge from the open side of the calyx. The tips of the two club-shaped enations bear stigmatic papillae. ×4·8.



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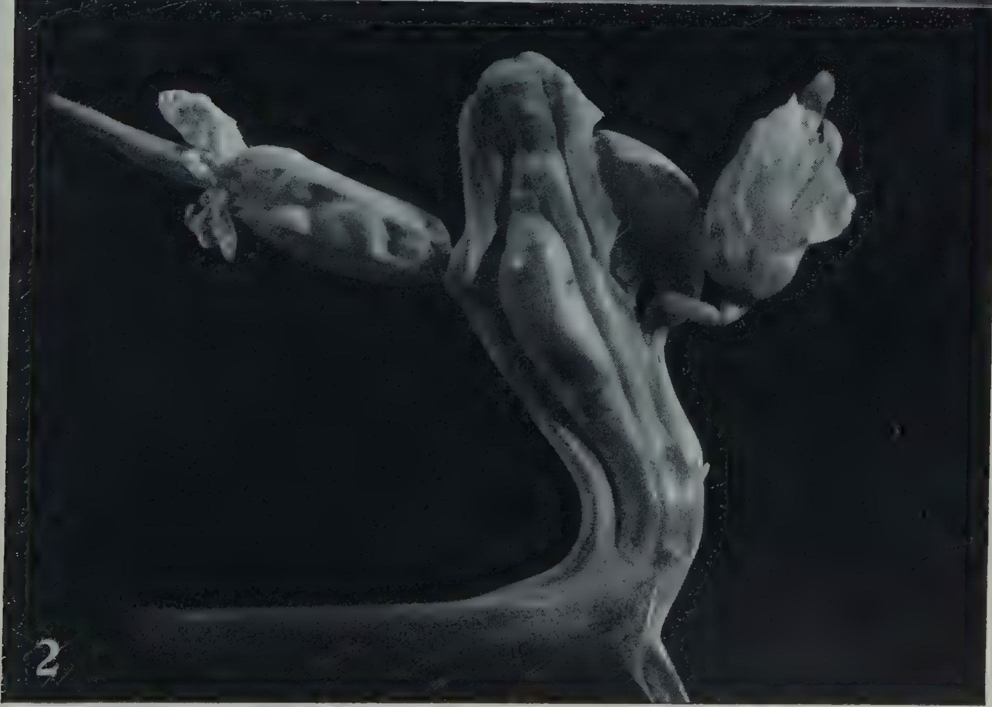

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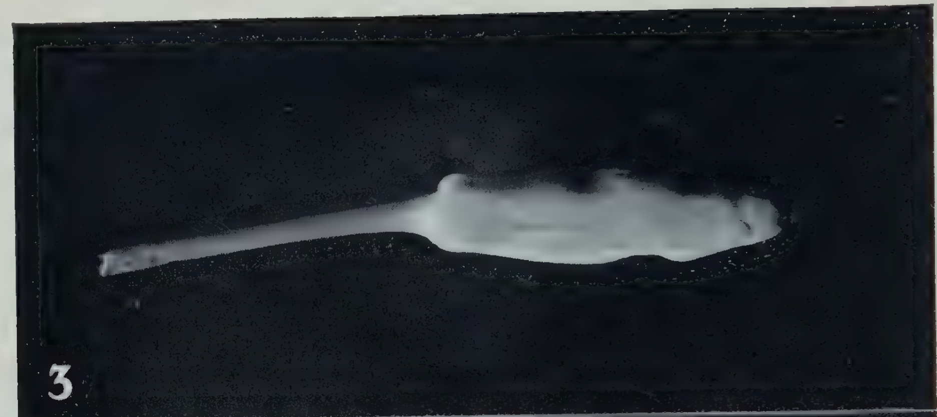

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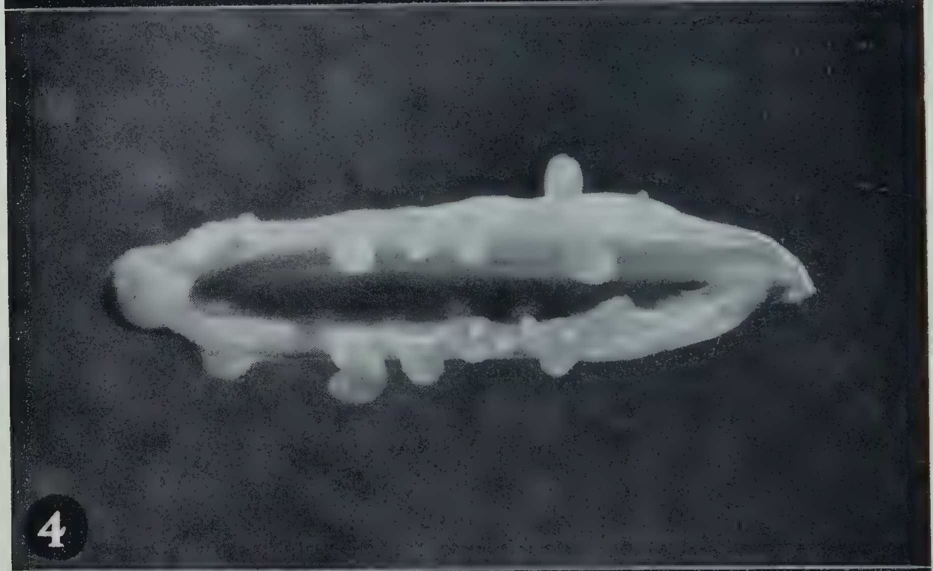

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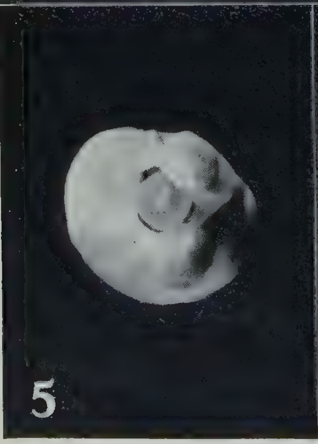

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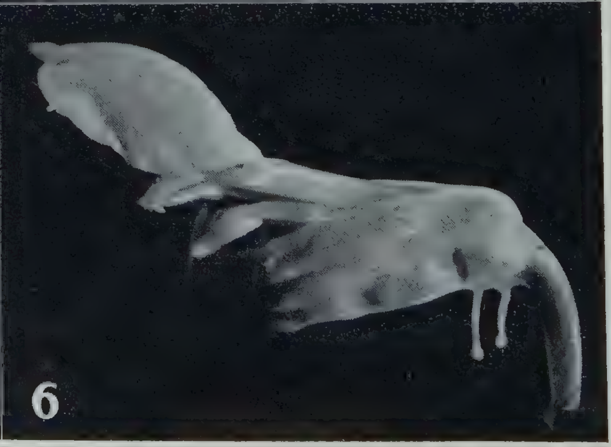

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D. R. SAMPSON and M. MacARTHUR

Inflorescence Initiation and Leaf Size in Some Gramineae

BY

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With six Figures in the Text

ABSTRACT

The morphology of successive leaves on the flowering shoot was studied in species of *Glyceria*, *Lolium*, and *Triticum*. The blades of successive leaves were progressively longer, eventually reaching a maximum, after which the blades of the last few leaves produced before heading were shorter. When the longest leaf blade was elongating, dissection of the shoot apices showed that inflorescence initiation was taking place. Epidermal cell measurements in *Triticum* indicate that differences in blade length are due to differences in the amount of cell extension.

It appears that a correlated change occurs in blade morphology associated with the onset of the reproductive state of the shoot apex, brought about via changes in the amount of cell extension.

A study of the effect of different amounts of low-temperature and different day-lengths on the relation between inflorescence initiation and the production of the longest leaf blade showed that, under some conditions, this relation can be disturbed.

INTRODUCTION

THE object of this paper is to present the results of studies on the morphology of successive leaves on the flowering shoot as influenced by inflorescence development in the Gramineae. Species of *Glyceria*, *Lolium*, and *Triticum* were investigated.

The data recorded include lengths, and in some cases widths, of successive leaf-blades, also the stage of inflorescence development on the main shoot. The timing of inflorescence development was altered experimentally by controlling the low temperature and photoperiod exposure given to the material.

When low temperature was given, the seeds were moistened with water for 12 hours, and then transferred to a refrigerator at between 0 and 5° C. Details of the material used and treatment applied are given in Table 1.

The seedlings were grown in boxes of sterilized soil and top dressings of fertilizer were applied as required to prevent nitrogen starvation.

Each leaf was measured when it had stopped growing, which was by the time half of the next leaf was visible, and its tip cut for future identification. The use of the appearance of successive leaves as a time-scale has been suggested by Mitchell (1953) and Cooper (1956).

In the *Lolium temulentum* material the shoot apex in three plants was dissected at weekly intervals. In Squarehead's Master wheat 30 plants were grown, and of these 15 were used for leaf measurements, and the remainder

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TABLE 1

Material and Treatments

Experiment no.	Species	Station number	Origin	No. of plants	Pre-treatment (weeks)	Date sown	Site	Photoperiod
1.	*Glyceria declinata* Breb. (inbreeding perennial)	Bs 2588	Lingwood Pond, Norfolk	22	Nil	2.3.53	Cold greenhouse	Natural day
		Bs 2589	Ashstead Woods, Surrey	22	,,	,,	,,	
		Bs 2590	Esher Common, Surrey	22	,,	,,	,,	
2.*	*Lolium perenne* L. (outbreeding perennial)	S.23 L.W.45	Aberystwyth-bred rye-grass strain	20	,,	14.1.50	Laboratory	Continuous fluorescent only
		S.24 B.M.45	Aberystwyth-bred rye-grass strain	20	,,	,,	,,	
	Lolium italicum A. Br. (outbreeding biennial)	Bb 409	English leafy Italian	20	,,	,,	,,	
	L. temulentum L. (inbreeding annual)	Ba 3081	Inbred line	15	,,	28.11.49	Cold greenhouse then outdoors	Natural day
				15	,,	9.11.49	Heated greenhouse	
				15	,,	,,	Unheated greenhouse	Continuous light (natural day plus fluorescent)
3.	*Triticum vulgare* L. var. Squarehead's Master (inbreeding annual)	Ca 942	From N.I.A.B. Cambridge	15	,,	10.1.56	Unheated greenhouse	Continuous light (natural day plus fluorescent)
				15	2	,,	,, ,,	
				15	4	,,	,, ,,	
				15	6	,,	,, ,,	
				15	8	,,	,, ,,	
				15	10	,,	,, ,,	
4.	*Triticum vulgare* L. var. Squarehead's Master (inbreeding annual)			15	Nil	13.8.56	Unheated, but later heated	Continuous light (fluorescent)
				15	,,	,,	,, ,,	17-hour day (natural daylight, plus fluorescent)
				15	3	,,	,, ,,	
				15	6	,,	,, ,,	8-hour day (natural daylight)
				15	3	,,	,, ,,	
				15	6	,,	,, ,,	

* Data collected by J. P. Cooper

for dissection. Epidermal cell lengths were measured, 12 cells were taken from the upper epidermis at the widest part of the leaf, using the band of cells between the second and third nerves away from the margin.

In the first wheat experiment, cell and leaf lengths were measured on leaves of corresponding number but from different plants, and in the second experiment measurements were made on the same leaf.

EXPERIMENTAL RESULTS

Temperature conditions varied between experiments, and sometimes between treatments carried out at different places and seasons of the year; the experiments therefore will be discussed separately.

Experiment 1, to compare the length and width of successive leaves on the main shoot in three lines of the perennial Glyceria declinata *from different localities.*

Fig. 1 shows that a similar curve was obtained for leaf length in each race, the flag-leaf being very short. Leaf width fluctuated much less in relation to position on the flowering shoot. Statistical comparisons were made between the flag leaves and the longest leaves, using a method described by Mather (1949).

Dimensions of leaves in mm.

	Bs 2588		Bs 2589		Bs 2590	
	Length	Width	Length	Width	Length	Width
Longest leaf . . .	181**	5·6	152	5·3	143	5·9
Flag-leaf. . . .	45	4·7*	42	5·3	48	5·2

Significantly different from the rest at:

$* = P = 0{\cdot}05$

$** = P = 0{\cdot}01$

Bs 2588 was significantly different from the other two in the longest leaf, whereas the flag-leaves were similar. Flag-leaf width was just significantly different in this sample mainly because this race produced one leaf more than the others before heading.

Experiment 2, to compare the leaf-length curves obtained for Lolium *spp. grown without low-temperature treatment.*

These are shown in Fig. 2; all display the same general trend within which there is a relation between shape of curve and rapidity of heading.

In the inbreeding annual *L. temulentum* the plants were dissected to find the number of leaves which had been formed up to the time when the inflorescence was initiated. This occurred in natural day-length between the time when the longest leaf became visible and its maturity. The longest leaf was the fifth in plants grown outdoors, and the sixth in those left in a heated house, corresponding to the peak of the leaf-length curves indicated by arrows in Fig. 2. In continuous light, the inflorescence was formed at the second leaf, early on the ascending part of the curve.

In these instances changes in the day-length were associated with a shift in the time of inflorescence initiation and a reduction in the number of leaves before heading.

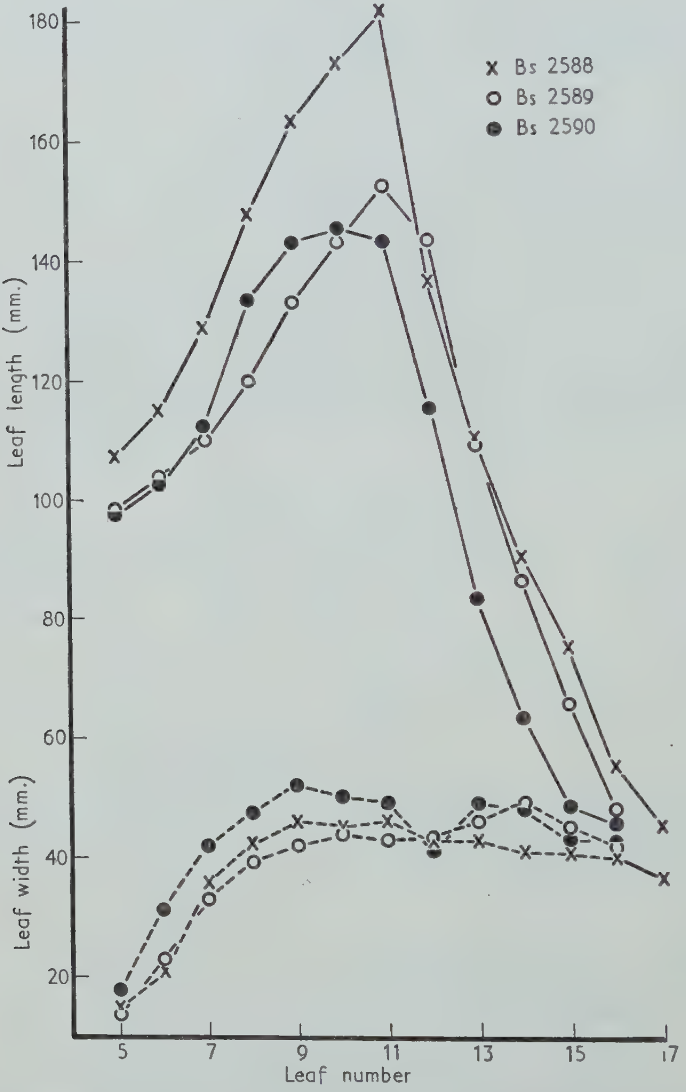

FIG. 1. *Experiment* 1. Length and width of successive leaves on the main shot of *Glyceria declinata.*

Experiment 3, to examine the effect of low temperature on leaf length in the winter wheat Squarehead's Master, sowings were made on 10 January 1956.

The grains were soaked in water for 12 hours to initiate germination and then subjected to the six low-temperature treatments shown in Table 1. In each treatment inflorescence initiation occurred at, or very near, the peak of the leaf-length curve, Fig. 3, which corresponds to the behaviour of *L. temulentum* in natural daylight.

Fig. 4 shows the effect of progressive increase in amount of low temperature applied to the soaked seed. There is a highly significant regression on low temperature in respect of the interval from germination to heading, number of leaves on the main shoot before heading, and number of leaves expanded when inflorescence initiation occurred. The regression of leaf length on epidermal cell length was not significant, possibly because the measurements were not made on the same leaf.

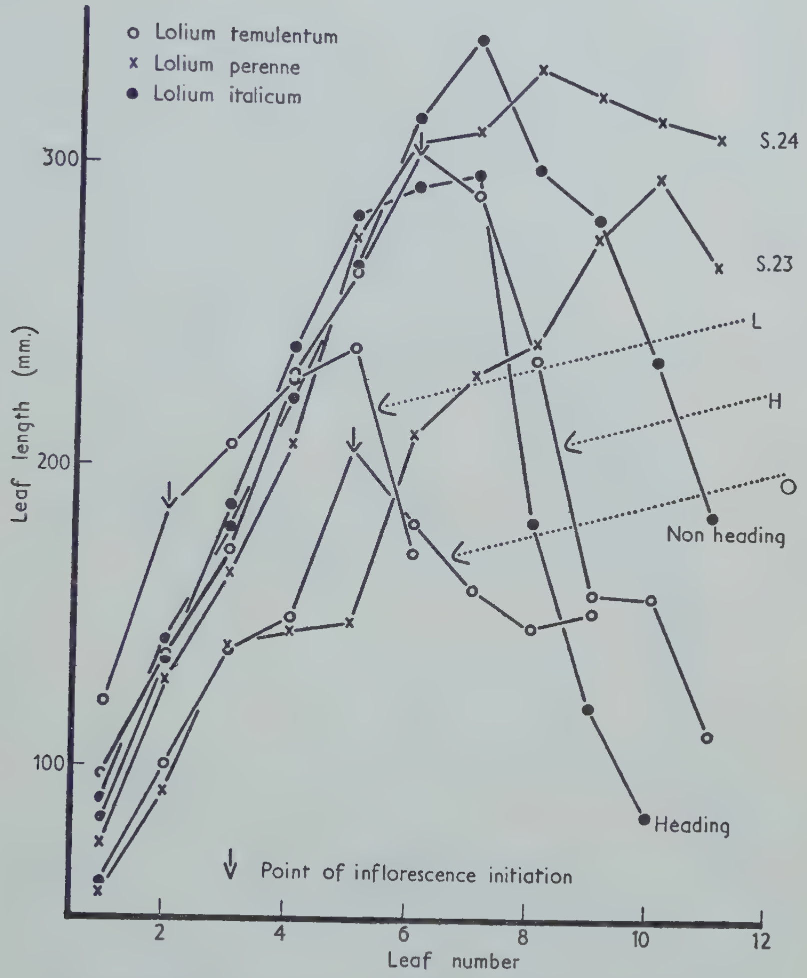

FIG. 2. *Experiment* 2. The length of successive leaves on the main shoot in *Lolium* species. o *L. temulentum*; ● *L. italicum*; X *L. perenne*, two strains, 523 and 524. L = continuous light; H = heated house, normal day-length; O = outdoor, normal day-length.

Experiment 4, to study the effect of low temperature and photoperiod on leaf length in Squarehead's Master wheat.

Sowings were made on 13 August 1956; three photoperiods were used, continuous light, 8-hour day, and 17-hour day, Table 1. Within each light-treatment 21 and 42 days of low temperature were given with *nil* low-temperature as a control. No data were collected for the 8-hour day no low-temperature treatment because of poor germination.

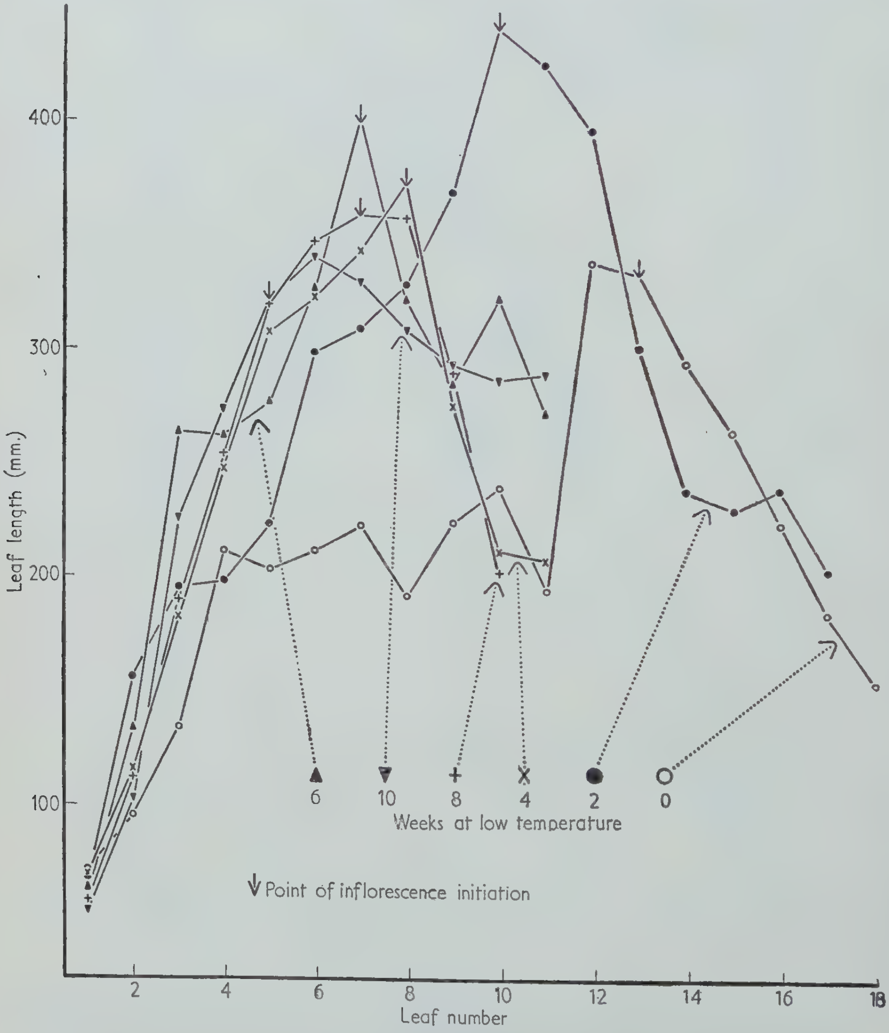

FIG. 3. *Experiment* 3. The effect of low-temperature treatment on the length of successive leaves on the main shoot, in Squarehead's Master wheat (Spring sown).

The resultant curves, Fig. 5, show that the response under continuous light and no low temperature was quite different from that observed in Experiment 3, in that inflorescence initiation did not occur at the peak of the leaf-length curve, but much later, associated with a second increase in leaf length. Near the beginning of this increase, from the twelfth leaf onward (5 November 1956), there was a slowing down in the rate of leaf appearance, from a mean of one every 7·2 days to one every 17·2 days.

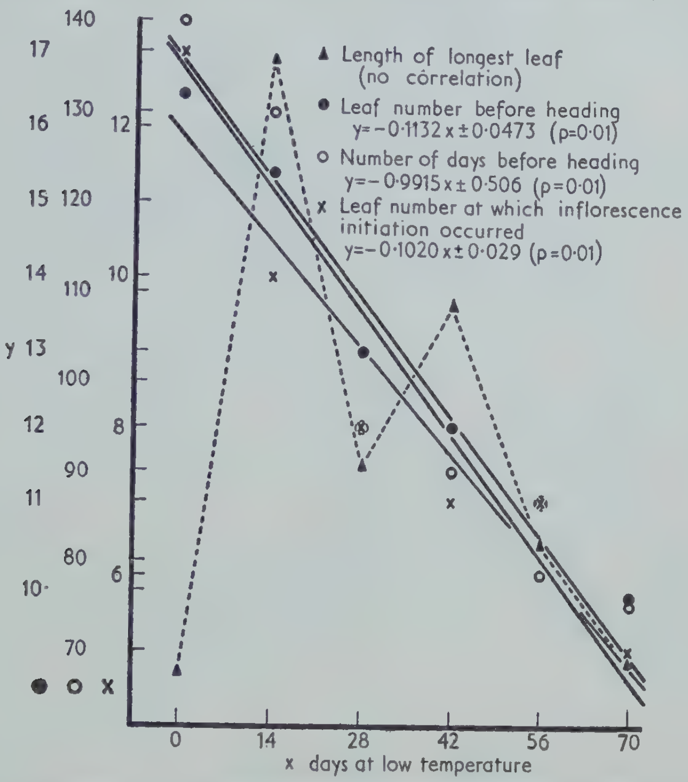

FIG. 4. *Experiment* 3. The regression of developmental response on duration of low temperature in Squarehead's Master wheat (Spring sown).

Only under 17-hour day and 42 days of low temperature was the curve of leaf length similar to that obtained throughout the third experiment. Day-length appears to have a primary effect on the general slope of the curves. Within the grouping by day-length, low temperature exerts an influence which seems to intensify the main changes on leaf length as follows:

Day-length (hrs.)	Days in low temp.	Changes in leaf length
Continuous	None	Steeply ascending then falling
17	42	Steeply ascending then falling
17	21	Steeply ascending, falling, then rising
17	None	Steeply ascending, falling, then rising
8	42	Gradually and irregularly ascending
21	21	Gradually and irregularly ascending

Cell-length measurements were made on the leaves of two plants from each treatment. The regression of leaf length on cell length was calculated as

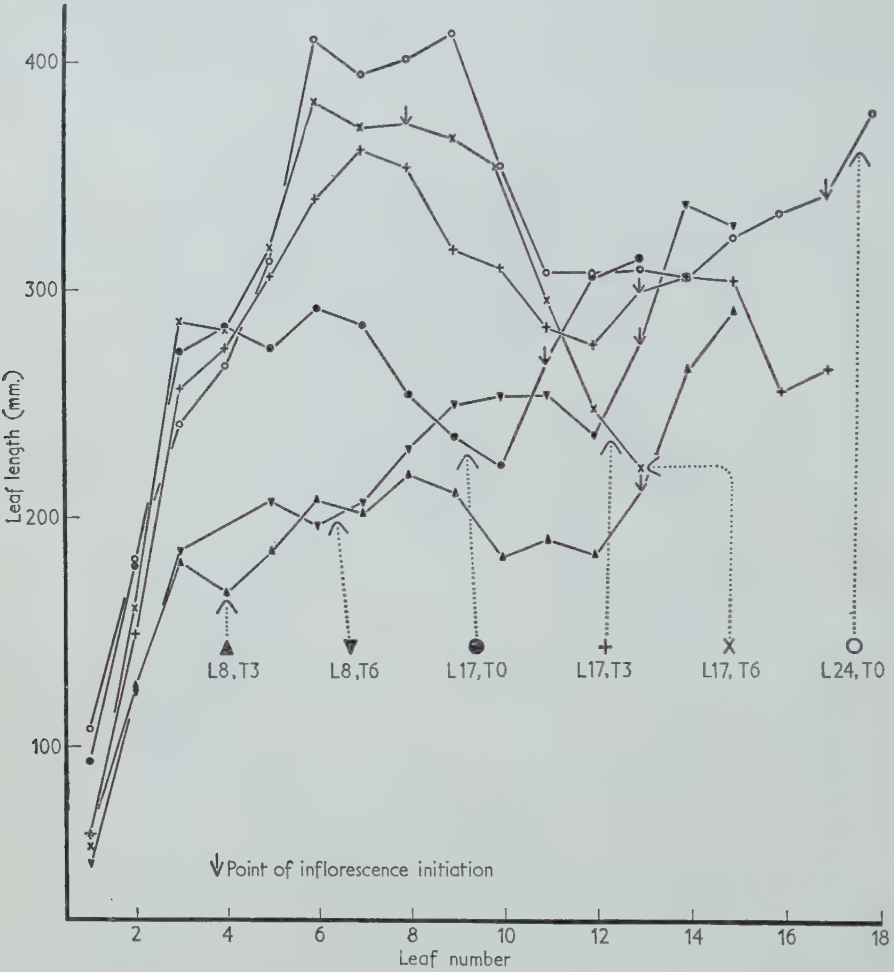

FIG. 5. *Experiment* 4. The effect of day-length and low-temperature treatments on the length of successive leaves on the main shoot in Squarehead's Master wheat (Autumn sown). L = hours of daylight; T = weeks at low temperature.

follows: (*a*) for all the data, (*b*) for leaves belonging to the part of curves where the length of successive leaves was increasing; and (*c*) for leaves belonging to part of the curves where the length of successive leaves was decreasing. In each case a highly significant regression was obtained from which it may be concluded that the extension of leaf blades is mainly brought about by cell elongation, Fig. 6. The small difference observed between the regression for leaves on the ascending and descending parts of the leaf-length curve suggests that for a given cell length, leaf length is slightly smaller in the first case and greater in the second.

DISCUSSION

It is clear from the results that the curve obtained for the length of successive leaves along the main shoot in the Gramineae studied is often of a definite type, namely, increasing to a peak and then falling until the flag-leaf is reached. This is shown in Figs. 1, 2, 3, and 5 for the following species and treatments:

Glyceria declinata	Natural day-length
Lolium italicum	Continuous light
Lolium temulentum	Continuous light; natural day-length outdoors, and in heated house
Spring-sown wheat	Continuous light
Autumn-sown wheat	17-hour day and 42 days of low temperature

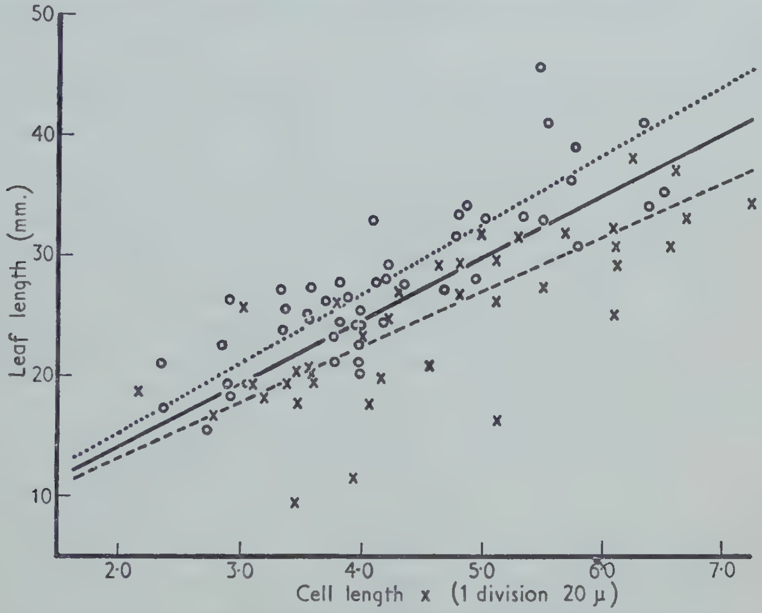

FIG. 6. *Experiment* 4. The regression of leaf length on cell length in Squarehead's Master wheat (Autumn sown). For all leaves (continuous line), $y = 5{\cdot}25x \pm 0{\cdot}29$; during period of length increase (broken line), $y = 4{\cdot}66x \pm 0{\cdot}46$; during period of length decrease (dotted line), $y = 5{\cdot}85x \pm 0{\cdot}51$.

It seems reasonable to suggest that in cereals and herbage grasses growing under field conditions this is the usual succession of blade length on flowering shoots during the season of active growth. Confirmatory evidence comes from observations made by Beddows (unpublished) on *Dactylis* and *Holcus* species in which it was found that the leaves on the panicle stem tend to increase in length from the flag-leaf downwards.

The data show that inflorescence initiation takes place when the longest blade is elongating and that the blades which appear subsequently are progressively shorter. Further, the wheat data suggest that differences in blade length are due to differences in the amount of cell extension and not to fluctuations in cell number. Avery (1934) found a similar situation in tobacco.

It appears that, with the onset of the reproductive state in the shoot apex, a correlated change occurs in blade morphology associated with variation in the amount of cell extension.

In *Lolium* spp. there is evidence (Cooper, unpublished) suggesting that in contrast to the curve described above for leaf length along the main shoot, the leaf sheaths become progressively longer, that of the flag-leaf being longest, and related to the degree of internode extension in the flowering shoot.

The existence of this trend of leaf length along the flowering shoot is of interest to the taxonomist, since the leaf is a useful homotype in distinguishing between races (Borrill 1957). The leaf-length trend clearly parallels the seasonal trend in total leaf area given for wheat and barley by Watson (1947); and it seems likely that leaf length on the individual culm is a component of this trend.

The effect of environmental factors on the time of inflorescence initiation and the slope and shape of the leaf-length curve can now be considered.

In *Lolium temulentum* (Fig. 2) the curve is of the same type for plants grown in a heated house as for those outdoors but, in continuous light, inflorescence initiation is advanced, occurring on the ascending part of the curve.

With spring-sown winter wheat (Fig. 3) increasing amounts of low temperature affect the slope of the ascending limb of the curve, reduce the interval to inflorescence initiation, and the number of leaves on the main shoot before heading. The relation in this case is a linear one (Fig. 4). The general slope of the curve and the correspondence in time of inflorescence initiation with the appearance of the longest leaf are maintained.

The behaviour of the autumn-sown winter wheat was interesting since it revealed an over-all seasonal effect. This is shown mainly by a difference in the general shape of the curve in relation to inflorescence initiation, which was delayed beyond the peak of the curve, to the early part of a second increase in leaf length, associated with a decrease in the rate of leaf production.

The differences in ascending slopes of the curves can be related to differences in day-length and low temperature, and in that order, whereas delay in time of inflorescence initiation cannot.

Two principal factors may affect the time at which the inflorescence was initiated, the difference in temperature conditions between the spring and autumn sowings, and the difference in total amount of light received. While the possible effects of these cannot be separated on the present data, it may be noted that in *L. temulentum*, continuous light altered the relation between inflorescence initiation and leaf-blade length. This may be due to the total amount of light received rather than photoperiod *per se*.

These studies are continuing and will be reported upon in a subsequent paper.

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The Circulation of Minerals in Plantations of *Pinus sylvestris* L.

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With three Figures in the Text

ABSTRACT

The dry weights and amounts of sodium, potassium, calcium, magnesium, phosphorus, and nitrogen in the ground flora and organic layers formed over the mineral soil are recorded for an age series of plantations of *Pinus sylvestris*. From these data, the distribution of mineral nutrients within the plantations is determined and calculations are made of the annual circulation of nutrients through the ecosystem. After 55 years of afforestation the amount of organic matter accumulated over the forest floor is only equivalent to the litter fall of the last 6 years. Thus, although afforestation results in a progressive accumulation of organic matter, the woodland community is characterized by a large and rapid transfer of nutrients between the plants and mineral soil.

INTRODUCTION

SJÖRS (1955) defined the ecosystem as 'The whole complex of organisms and factors of environment in an ecological unit'. A forest plantation composed of a single tree species may be regarded as a simple ecosystem in which the development of its components, both living and dead, is influenced by the intensity of silvicultural management and the natural changes that occur as a woodland matures. The inter-relationships between the trees and other factors of the ecosystem are complex but, by investigating an age series of plantations, data can be obtained to illustrate some of the interactions within woodland communities and the differences in mineral circulation at different stages of development.

In two previous papers (Ovington, 1957 and 1959) data were given of the dry weights and mineral contents of the trees from an age series of plantations of *Pinus sylvestris* at Thetford Chase. This account is concerned with the associated changes in the ground flora and in the organic layers formed over the mineral soil of the plantations.

METHODS

Sixteen quadrats (25 × 25 cm. square) were marked out in each plantation, four quadrats being located at random along each of four parallel, evenly spaced lines across the sample plot. The ground flora above ground level and

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the layer of organic matter over the mineral soil in each quadrat were collected. The organic matter was separated into an upper horizon (L) of only slightly altered plant remains, a central horizon (F) of more decomposed litter, e.g. small leaf fragments and incomplete cone scales, and a bottom horizon (H) of amorphous humus. The upper litter horizon (L) was further subdivided according to whether it originated from the ground flora, pine leaves, cones, or branches. The bulk of any small animals present in the litter would be included within the F and H horizons.

After separation, the ground flora and litter samples were oven dried at 80° C. and weighed. The dried plant material was then ground to a fine powder and analysed for various chemical elements using the same methods as for the trees.

The ground flora and litter samples were collected in October 1956, 2 years after the trees were sampled, but since the plantations form an age series it has been possible to estimate the weights of vegetation and litter in the plots at the time the trees were sampled.

THE GROUND FLORA

Watt (1936) described the characteristic soil types and associated plant communities of the open Breckland heaths. The variation in soil properties over the area in which the sample plots are located suggests that there would have been some inherent differences in the plant cover of the plots before afforestation began. Differences in the abundance of *Calluna vulgaris* are probably of the greatest significance in considering the circulation of minerals because of the tendency for surface humus to form under heather and because of the perennial and woody nature of heather which results in a continued accumulation of nutrients within the living plant mass.

TABLE I

The Weight and Mineral Content (kg./ha.) of the Ground Flora in the Pine Stands

Tree age (years)	Oven-dry weight	Sodium	Potassium	Calcium	Magnesium	Phosphorus	Nitrogen
7	11,582	1·51	23·28	23·51	6·02	7·88	113·62
11	7,395	1·15	20·36	25·93	5·34	7·00	71·26
14	2,229	0·56	11·79	19·07	3·17	4·16	20·19
17	296	0·08	0·67	1·08	0·23	0·30	3·65
20	261	0·04	0·85	1·65	0·19	0·37	4·14
23	152	0·15	1·75	1·24	0·30	0·25	2·12
31	1,558	1·08	21·59	12·44	2·01	2·22	17·74
35	1,969	0·82	18·06	12·64	2·01	2·77	24·92
55	2,644	3·73	22·27	19·33	5·43	3·24	39·76

Despite any initial differences between plots, progressive changes in the ground flora can be recognized and are associated with changes in the intensity of tree cover (Table 1). At first, when the trees are small and the plantations are kept free from grazing animals such as rabbits, there is a varied and heavy ground flora. This ground flora has almost completely disappeared from the dense 17-year-old plantation and only a few small patches of moss remain. Forest thinning begins at about 20 years of age and after 23 years a new ground flora gradually becomes established until in the oldest plantation there is a rank growth of bracken with an understory consisting mainly of *Holcus mollis* and *Rubus fruticosus*. Despite the low stocking of the 55-year-old plantation, the trees are still apparently having a repressive effect on the ground flora which never becomes so luxuriant, in terms of dry weight, as in the younger plots (Fig. 1).

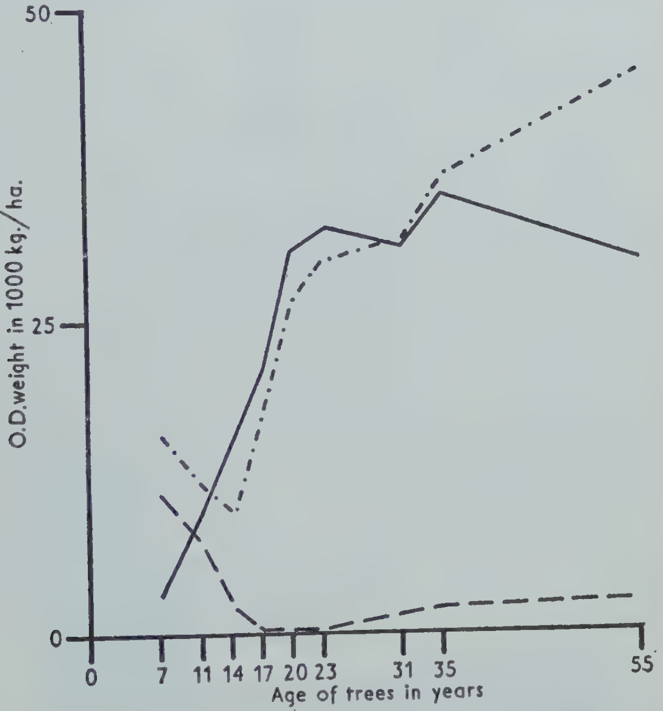

FIG. 1. The oven-dry weights of the tree canopies (——), surface organic matter (–·–·–) and ground flora (— — —) in plantations of *Pinus sylvestris*.

When well developed, the ground flora contains fairly large amounts of plant nutrients, the amount depending on the dry weight and percentage composition. The gradual killing and decomposition of the ground vegetation with the change from open heathland to dense, rapidly growing pine woodland releases significant amounts of plant nutrients for tree growth during the critical period of high forest productivity and nutrient absorption. In contrast, the spread and development of the bracken vegetation in the older pine stands reflect an increasing competition between the trees and ground flora for the available nutrients and water. At this stage, when the plantations are approaching maturity, the annual dry weight increment of the tree stock is relatively small.

SURFACE ORGANIC MATTER

The layer of organic matter over the surface of the mineral soil of the plantations is derived mainly from litter falling from the trees and ground flora but also contains roots and soil micro-organisms. In general, the total weight of surface organic matter increases throughout the 55-year period although the rate of increase begins to decline towards the end. The amounts of fresh litter (L) and partially decomposed litter (F) tend to increase rapidly during the first fourteen to seventeen years and then remain fairly constant until the bracken begins to invade the older plantations (Table 2). The bracken litter apparently decomposes relatively slowly since a thick, loose layer of bracken rachides soon accumulates. After the initial build-up period, the continued formation of an organic layer over the ground results mainly from the accumulation of amorphous organic matter, suggesting that breakdown of the litter is delayed during the latter stages of decomposition. The youngest plantation is unusual in that there is a relatively large proportion of amorphous organic matter due to the exceptional abundance of heather in the ground flora of this plot.

TABLE 2

The Development of the Surface Organic Layer (O.D. weight kg./ha.) in the Pine Stands

	Fresh litter, horizon (L)					
Tree age (years)	Pine needles	Pine twigs	Pine cones	Ground flora	Partially decomposed litter, horizon (F)	Amorphous horizon (H)
7	231	0	0	2,434	0	13,317
11	1,006	2	81	1,420	289	9,346
14	1,834	7	255	574	1,221	5,984
17	1,747	16	633	718	2,635	11,632
20	1,336	50	941	248	2,794	21,001
23	1,503	310	981	66	2,717	24,190
31	1,262	602	821	113	1,858	26,871
35	1,340	419	889	83	1,790	32,173
55	1,192	586	1,402	887	3,038	37,906

The changing proportions of the components of the L horizon reflect the varied quantities and types of litter fall at different stages of development (Fig. 2), as well as the differential rates of decomposition. For example, the annual production and shedding of pine leaves in the plantations becomes fairly constant after 11 years, when the tree canopies have closed, and the weight of pine needles in the L horizon does not change greatly thereafter. Consequently, from 11 years onwards, the initial processes of needle decomposition are keeping pace with the annual needle fall. In contrast, the weights of pine twigs and cones in the L horizon continue to increase until the plantations are about 23 years of age because, compared with leaves, the branches and cones decompose relatively slowly and the amount of branch and cone material falling to the ground tends to increase over a longer period. The sudden increase in the weight of twigs in the litter after 20 years is a result of the first heavy thinning when the canopies of the harvested trees are left to decompose in the plots.

Large quantities of surface organic matter are accumulated in the older pine plantations and, as the litter cover becomes thicker and heavier, it contains increasing amounts of plant nutrients so that humus formation is continually associated with the increasing immobilization of nutrients which might otherwise have been available for plant growth (Table 3). The dry weight of the surface humus is broadly equivalent to that of the living canopy of the trees (Fig. 1) and a clear felling in which the canopy was left to decompose on the ground would double the quantity of surface humus. Of course, this increase would only be temporary for the annual supply of litter from the trees would end with clear felling and decomposition may be stimulated in the cleared areas.

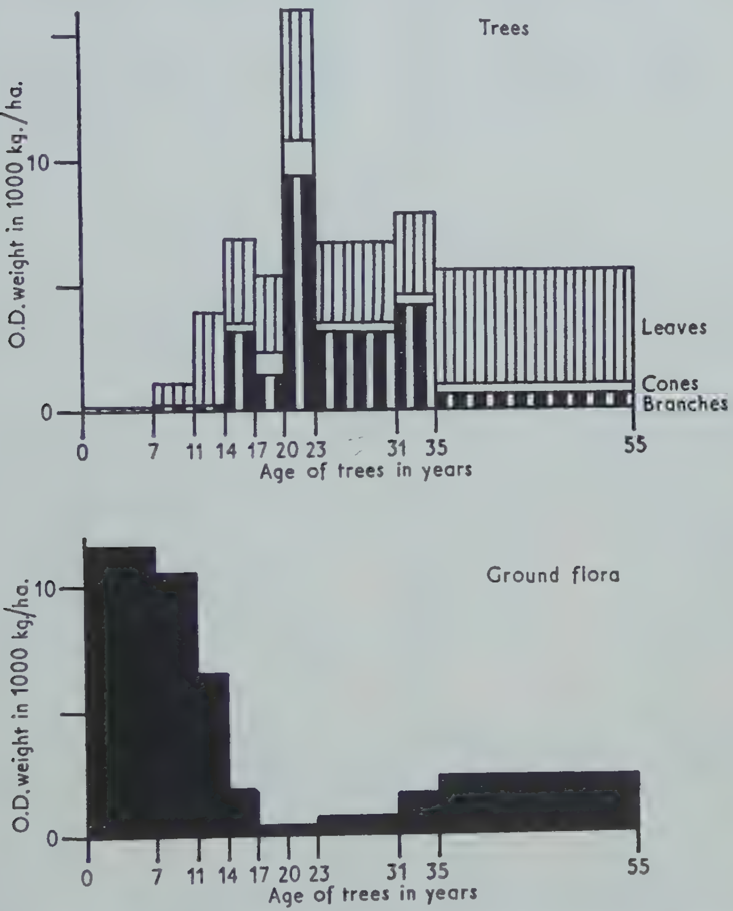

Fig. 2. The annual litter fall from the trees and ground flora in plantations of *Pinus sylvestris*.

The accumulative weight and nutrient content of the litter falling from the trees and ground flora are given in Table 4. The data for the trees are based on the differences between the gross production of leaves, cones, and branches

TABLE 3

The Weight and Mineral Content (kg./ha.) of the Surface Organic Matter in the Pine Stands

Tree age (years)	Oven-dry weight	Sodium	Potassium	Calcium	Magnesium	Phosphorus	Nitrogen
7	15,982	0·84	10·02	26·19	8·59	8·97	158·89
11	12,144	1·08	10·95	47·48	6·97	7·13	116·64
14	9,875	1·22	11·71	63·27	5·76	6·76	90·71
17	17,381	1·57	15·51	77·11	7·87	13·49	193·86
20	26,370	2·57	23·29	127·46	11·43	21·04	336·03
23	29,790	2·89	24·29	138·89	16·93	21·92	162·89
31	31,527	3·79	31·48	122·54	16·27	19·07	285·19
35	36,694	4·53	31·16	128·02	26·77	21·99	345·79
55	45,011	6·47	34·38	141·40	30·71	27·56	408·72

TABLE 4

Litter Fall and Decomposition

Age of trees (years)	7	11	14	17	20	23	31	35	55
Dry weight 1,000 (kg./ha.)									
Litter from trees	0·66	5·04	16·60	37·35	53·60	101·89	155·08	186·52	296·98
Litter from ground flora	69·49	111·64	131·24	136·96	137·83	138·56	144·00	150·64	196·11
Total litter fall	70·15	116·68	147·84	174·31	191·43	240·45	299·08	337·16	493·09
Decomposed	54·17	104·54	137·96	156·93	165·06	210·66	267·55	300·47	448·08
Sodium (kg./ha.)									
Litter from trees	0·7	2·4	4·5	6·1	7·4	11·4	22·0	25·8	48·6
Litter from ground flora	9·0	14·7	17·9	19·3	19·5	19·7	23·7	27·8	70·4
Total litter fall	9·7	17·1	22·4	25·4	26·9	31·1	45·7	53·6	119·0
Released by decomposition	8·9	16·0	21·2	23·8	24·3	28·2	41·9	49·1	112·5
Potassium (kg./ha.)									
Litter from trees	1	21	73	136	225	410	687	847	1,593
Litter from ground flora	140	230	287	316	319	322	395	478	877
Total litter fall	141	251	360	452	544	732	1,082	1,325	2,470
Released by decomposition	131	240	348	436	521	708	1,051	1,294	2,436
Calcium (kg./ha.)									
Litter from trees	9	37	133	247	304	470	774	923	1,579
Litter from ground flora	141	238	312	360	364	368	412	462	775
Total litter fall	150	275	445	607	668	838	1,186	1,385	2,354
Released by decomposition	124	228	382	530	541	699	1,063	1,257	2,213
Magnesium (kg./ha.)									
Litter from trees	<1	4	21	33	47	84	143	171	288
Litter from ground flora	36	60	74	83	83	84	91	99	170
Total litter fall	36	64	95	116	130	168	234	270	458
Released by decomposition	27	57	89	108	119	151	218	243	427
Phosphorus (kg./ha.)									
Litter from trees	<1	5	17	37	57	102	152	185	325
Litter from ground flora	47	78	97	108	109	110	118	127	187
Total litter fall	47	83	114	145	166	212	270	312	512
Released by decomposition	38	76	107	132	145	190	251	290	484
Nitrogen (kg./ha.)									
Litter from trees	3	51	235	430	638	1,039	1,675	1,983	3,863
Litter from ground flora	682	1,094	1,282	1,334	1,346	1,357	1,421	1,499	2,131
Total litter fall	685	1,145	1,517	1,764	1,984	2,396	3,096	3,482	5,994
Released by decomposition	526	1,028	1,426	1,570	1,648	2,233	2,811	3,136	5,585

and the weights of the living stock. The ground flora values, which have been obtained by assuming that the aerial parts of the herbaceous plants fall as litter each year, will give an overestimate if woody perennials are present and if there is any translocation of nutrients to the subterranean organs prior to the death of the shoots. Initially, the ground flora contributes more to the litter fall than the trees but gradually as the minor vegetation is suppressed more litter is shed from the trees than the ground flora.

The amount of decomposition that has taken place was calculated by subtracting the weight of surface organic matter from the accumulative litter fall. When the trees are 55 years of age about 91 per cent. of the litter that has fallen has decomposed and there has been a corresponding release of plant nutrients so that a very effective interchange of nutrient elements takes place between the woodland plants and mineral soil. The surface organic matter present after 55 years of tree growth is broadly equivalent to the amount of litter falling in the last 6 years. Nevertheless, because of the differential rates of decomposition of the organic matter the litter will contain plant remnants which have been on the ground more than 6 years.

THE IMMOBILIZATION AND CIRCULATION OF NUTRIENTS

Despite the large annual fall of litter from the trees and ground flora, the conversion of the Breckland heaths to actively growing pine woodlands results in a progressive increase in the total weight and mineral content of the living plants and organic layers throughout the 55-year period (Table 5). The build-up of organic matter within the ecosystem and the interchange of nutrients between the plants and mineral soil are affected by various factors which differ in magnitude as the pine stands mature and in Table 6 an attempt has been made to summarize the woodland dynamics at different stages of development.

TABLE 5

The Build-up of Organic Matter and Accumulation of Minerals Within the Trees (inclusive of Harvested Boles), Ground Flora, and Litter

Age of trees (yrs.)	Dry weight 1,000 kg./ha.	Sodium kg./ha.	Potassium kg./ha.	Calcium kg./ha.	Magnesium kg./ha.	Phosphorus kg./ha.	Nitrogen kg./ha.
7	35·02	3·3	59	68	20	26	342
11	45·52	5·2	97	236	30	32	370
14	45·42	5·8	99	164	25	40	324
17	65·79	4·2	151	168	34	51	499
20	91·98	5·7	190	255	45	65	709
23	136·74	12·7	189	334	62	55	612
31	204·99	13·7	281	502	73	72	717
35	267·52	49·7	335	579	112	83	912
55	365·27	51·8	305	643	142	86	1,062

Although some of the differences between sampling periods may result from site variation, on average approximately 17,000 kilograms of dry matter are produced annually by the trees and ground flora in each hectare of plantation and this results in an absorption of about 3 kilograms of sodium, 50 of potassium, 55 of calcium, 11 of magnesium, 11 of phosphorus, and 126 of nitrogen (Fig. 3). In general, the amounts of minerals taken up by the woodland plants are broadly equivalent to the quantities absorbed by agricultural crops, the production of which is maintained by the regular application of fertilizers to the soil. Only a small part of the nutrients absorbed by the woodland plants is retained in the trees, ground flora, and litter but this represents a more or less continuous drain on the soil resources since most of the values for this given in Table 6 are positive. Where afforestation increases the mass and mineral content of the fauna, a further drain of nutrients may take place, but this is not likely to be so great as that associated with the build-up of the plant mass. The total mineral content of the canopy fauna (birds and insects) for each hectare of 23–30 year old plantations of Scots pine is only about 2 g. of sodium, 8 of potassium, and 8 of phosphorus (Grimshaw *et al.* 1958) and the calcium, magnesium, and nitrogen contents probably only amount to 2, 3, and 56 g. respectively. It is doubtful if the mineral content of the rest of the larger fauna, mainly red squirrels, roe deer, rabbits, hares, stoats, small rodents, and pheasants greatly exceeds these values. The litter fauna would tend to be included in the litter samples. The passage of minerals through the fauna will be more rapid than through the plants but on a long-term basis the total circulation through the fauna is likely to be regulated by the plant uptake.

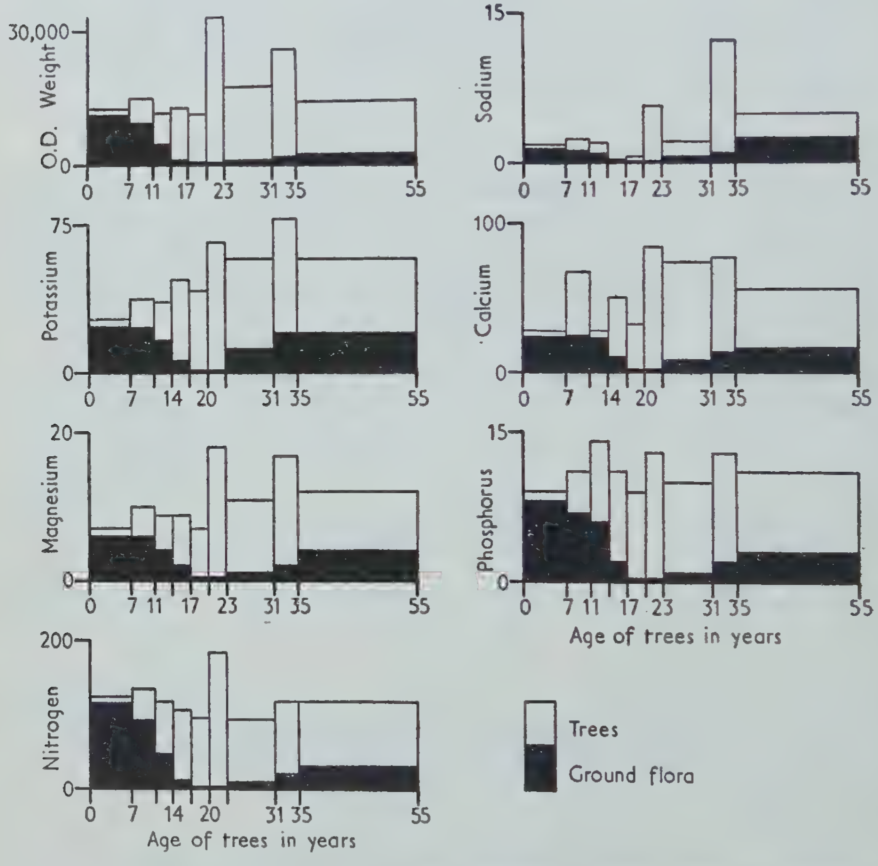

FIG. 3. Annual production of organic matter and uptake of nutrients (kg./ha.) by the ground flora and trees in plantations of *Pinus sylvestris.*

Only small amounts of nutrients are removed from the ecosystem in the harvested tree boles compared with those circulating through the system as a whole. The tree roots left in the ground after the trees are harvested contain

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TABLE 6. *Annual Balance Sheet for Plantations*

	Period (years)	0–7	7–11	11–14	14–17	17–20	20–23	23–31	31–35	35–55
Dry weight (1,000 kg./ha.)	Produced by trees and ground flora	12·74	15·19	11·18	13·06	11·45	33·14	17·20	25·96	14·20
	Returned to mineral soil by litter decomposition	7·74	12·59	11·14	6·32	2·71	15·20	7·11	8·23	7·38
	Left in mineral soil as roots of harvested trees	0	0	0	0	0	3·03	1·54	2·10	1·93
	Removed from ecosystem in boles of harvested trees	0	0	0	0	0	5·03	3·70	5·20	5·07
	Change in remaining trees, ground flora, and litter mass	+5·00	+2·63	−0·03	+6·79	+8·73	+9·89	+4·83	+10·43	−0·18
Sodium (kg./ha.)	Uptake by trees and ground flora	1·7	2·2	1·9	0·3	0·7	4·2	2·2	12·2	4·9
	Returned to mineral soil by litter decomposition	1·3	1·8	1·7	0·9	0·2	1·3	1·7	1·8	3·2
	Left in mineral soil as roots of harvested trees	0	0	0	0	0	0·6	0·4	1·3	1·6
	Removed from ecosystem in boles of harvested trees	0	0	0	0	0	0·1	<0·1	0·1	0·2
	Change in remaining trees, ground flora, and litter mass	+0·5	+0·5	+0·2	−0·5	+0·5	+2·2	+0·1	+8·9	−0·1
Potassium (kg./ha.)	Uptake by trees and ground flora	27	37	36	47	41	66	58	78	58
	Returned to mineral soil by litter decomposition	19	27	36	29	28	62	43	61	57
	Left in mineral soil as roots of harvested trees	0	0	0	0	0	4	3	3	2
	Removed from ecosystem in boles of harvested trees	0	0	0	0	0	4	2	3	3
	Change in remaining trees, ground flora, and litter mass	+8	+10	+1	+17	+13	−4	+10	+11	−5
Calcium (kg./ha.)	Uptake by trees and ground flora	28	68	28	51	32	84	74	77	56
	Returned to mineral soil by litter decomposition	18	26	51	49	4	53	46	49	48
	Left in mineral soil as roots of harvested trees	0	0	0	0	0	5	7	9	5
	Removed from ecosystem in boles of harvested trees	0	0	0	0	0	6	5	7	6
	Change in remaining trees, ground flora, and litter mass	+10	+42	−24	+1	+29	+20	+16	+12	−3
Magnesium (kg./ha.)	Uptake by trees and ground flora	7	10	9	9	7	18	11	17	12
	Returned to mineral soil by litter decomposition	4	8	11	6	4	11	8	6	9
	Left in mineral soil as roots of harvested trees	0	0	0	0	0	2	1	1	1
	Removed from ecosystem in boles of harvested trees	0	0	0	0	0	1	1	1	1
	Change in remaining trees, ground flora, and litter mass	+3	+3	−2	+3	+4	+5	0	+9	+1
Phosphorus (kg./ha.)	Uptake by trees and ground flora	9	11	14	11	9	13	10	13	11
	Returned to mineral soil by litter decomposition	5	10	10	8	4	15	8	10	10
	Left in mineral soil as roots of harvested trees	0	0	0	0	0	2	1	1	1
	Removed from ecosystem in boles of harvested trees	0	0	0	0	0	1	<1	1	<1
	Change in remaining trees, ground flora, and litter mass	+4	+2	+3	+4	+5	−4	+2	+2	+<1
Nitrogen (kg./ha.)	Uptake by trees and ground flora	124	134	118	106	96	183	93	138	139
	Returned to mineral soil by litter decomposition	75	126	133	48	26	195	72	81	122
	Left in mineral soil as roots of harvested trees	0	0	0	0	0	20	8	8	9
	Removed from ecosystem in boles of harvested trees	0	0	0	0	0	7	4	5	5
	Change in remaining trees, ground flora, and litter mass	+49	+7	−15	+58	+70	−39	+9	+44	+3

significant quantities of nutrients but, since the roots decompose slowly, the nutrients do not become quickly available to the surviving plants. The presence of root fusions suggests that some roots of harvested trees may survive and become part of the root system of the remaining trees.

DISCUSSION

The trees and ground flora are essentially complementary in that an increase in the luxuriance of one is usually associated with a decrease in the other. This combination of woody and herbaceous plants in the forest community enables the photosynthetic tissues to be dispersed throughout a large depth so that leaves of different shade tolerance and of varied forms occur in successive layers from the tree apices to the ground surface. In this way, the incident energy and available carbon dioxide can be effectively utilized for photosynthesis so that the community as a whole can attain high annual rates of dry-matter production. The woodland community is also capable of absorbing large amounts of water and minerals from a large soil volume since both deep-rooting tree species and more shallow-rooting herbs are present.

The trees and ground flora, as the two primary producers of the forest community, dominate and control the rest of the ecosystem through their influence on the micro-climate and biogeochemical cycles. Yet they are not completely independent, since the cycles would never be completed without the activities of the micro-organisms in breaking down the large litter fall and on occasion the balance within the ecosystem can be upset if some pest attains excessive numbers so as to throw the system out of balance (Elton, 1958).

Soil fertility is known to be affected by forest growth which may change the total amount and distribution of nutrients within the soil. Recently, increased attention has been paid to the danger of soil degradation as a result of tree growth and the removal of nutrients in the harvested tree boles. The general application of fertilizers, particularly of calcium, to forest soils has been advocated and may prove to be necessary but the effects of manuring should be considered in relation to the dynamics of the woodland community as a whole. For instance, it is obvious from the nitrogen content of the tree stock that large amounts of nitrogen are fixed annually in the soils of pine plantations but the long-term effects of calcium applications on nitrogen fixation as a result of the modification of the ground flora and litter are not clear. The woodland community is essentially a more natural community than agricultural crops and the large annual absorption of nutrients for tree growth and the circulation of organic matter and minerals through the system must ensure an active and a continuous mineralization of soil without necessarily increasing the loss of nutrients in the drainage water. Significant amounts of plant nutrients are known to be supplied to the woodland ecosystem in the precipitation and it does seem possible that the overall effect of the water cycle may be to supply nutrients to the upper soil and partially counterbalance any impoverishment due to nutrient absorption.

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Changes in Sensitivity to Inhibitors of Disks of Storage Tissue

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With six Figures in the Text

ABSTRACT

The respiratory sensitivity to cyanide of disks of sugar-beet, red beet, carrot, swede, and potato maintained at different temperatures over a prolonged period has been determined manometrically, and the effects of sodium azide and carbon monoxide examined on sugar-beet disks. The normally high sensitivity to cyanide of freshly cut disks falls more or less rapidly depending on the temperature of pretreatment until the oxygen uptake is practically unaffected by the inhibitor. Subsequently the sensitivity redevelops but a brief period during which cyanide exercises a stimulatory effect may be interposed. With beet and carrot disks containing cambial cells there is in the early stages of the lifespan a period of increasing sensitivity to cyanide. It is concluded that there is a characteristic difference in cyanide-sensitivity between growing and non-growing tissue. A discussion of underlying theory is added.

INTRODUCTION

EVIDENCE gleaned from inhibitor studies suggests that changes in metabolic rates of plant tissue are accompanied by changes in the terminal stages of oxidation. Alterations in sensitivity to various inhibitors have been reported to result from parasitic attack in wheat leaves (Király and Farkas, 1957); growth and differentiation in wheat roots (Eliasson and Mathiesen, 1956); wounding of beet disks (Robertson, Turner, and Wilkins, 1947); ageing of potato disks (Schade, Levy, Bergmann, and Harris, 1949; Thimann, Yocum, and Hackett, 1954) and chicory root slices (Laties, 1958); loss of growth potential in potato disks and etiolated pea internodes (Eichenberger and Thimann, 1957); ageing of carrot leaves (Marsh and Goddard, 1939), barley leaves (Merry and Goddard, 1941), mustard leaves, and dwarf shoots of larch (MacDonald and DeKock, 1958*a*); dormancy break and growth in potato tubers (Todd, 1953); germination and growth in oat seedlings (Albaum and Eichel, 1943), wheat seedlings (Waygood, 1950), barley seedlings (James and Boulter, 1955), and lettuce seeds (Mayer, Poljakoff-Mayber, and Appleman, 1957; Poljakoff-Mayber and Evenari, 1958). The progressively decreasing inhibition of oxygen uptake by heavy-metal inhibitors during the development and maturation of seedlings is generally regarded as evidence of a transition from sensitive to insensitive oxidases (James, 1953*a*).

Similar trends have been found in animal tissues. Unfertilized echinoderm

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eggs have a low respiration rate insensitive to cyanide in contrast to the rapid highly cyanide-sensitive respiration of fertilized eggs. Similarly during the embryonic development of the grasshopper, the egg 3 weeks after fertilization enters a dormant condition for several months in which the respiration rate is low and unaffected by cyanide or carbon monoxide, whereas the respiration both before and after the diapause is high and very sensitive to these inhibitors (Needham, 1942). Here cyanide-sensitive respiration appears to be associated with growth and development.

Among the factors which are thought to induce variation in terminal oxidase activity, James (1953*a*) lists both physiological development and the stress of experimental treatment such as slicing. The radical effect of slicing which causes a marked development of respiratory activity in storage tissue is well known and disks of some, if not all, storage tissue unquestionably possess the capacity for renewed growth. Some evidence was obtained previously that growth occurred in disks of sugar-beet tissue (MacDonald, 1955). Consequently the investigation of cyanide-sensitivity patterns in ageing disks of different storage organs was undertaken to discover what relationship existed between metabolic activity and cyanide sensitivity, it being considered that the cyanide-sensitivity patterns might show whether or not a development of metabolic activity leading to renewed growth and cell division was occurring in any of these tissues.

Materials and Methods

Disks from the storage organs of sugar and red beet (*Beta vulgaris*), carrot (*Daucus carota*), swede (*Brassica napus*), and potato (*Solanum tuberosum*) were prepared and maintained under uniform conditions as previously described (MacDonald and Knight, 1958). Oxygen uptake by the disks was measured by conventional Warburg manometry as in earlier experiments (MacDonald and DeKock, 1958*b*). Studies in cyanide inhibition were carried out using the centre-well solutions recommended by Robbie (1948). Daily, or at suitable intervals, the effect of cyanide on the respiration of the tissue was investigated over a 2-hour period. Occasional experiments prolonged over a 6-hour period demonstrated that the level of cyanide inhibition showed no further increase compared with the 2-hour period. Carbon monoxide experiments were conducted using the evacuation procedure described by Umbreit, Burris, and Stauffer (1947). All values recorded represent the average of at least 3 and often 4 replicates for each sample of tissue. The degree of inhibition or stimulation of oxygen uptake is expressed as a percentage of the control value and presented in graphical form in the text. Numerical values recorded for oxygen consumption are tabulated in an appendix. As in the previous paper, oxygen uptake is expressed on a 10-disk basis since neither the fresh weight nor dry weight of the tissue remains constant throughout the experimental period. Ten disks, 1·1 cm. in diameter and 0·1 cm. thick, weigh approximately 1·0 g. when freshly cut.

EXPERIMENTAL RESULTS

1. *Cyanide-sensitivity patterns in ageing disks*

(*a*) *Swede.* That the response of swede disks to cyanide inhibition changes drastically with age is evident from inhibition values obtained at 25° C. from disks maintained at 25° C. (Fig. 1A). Increasing the concentration of cyanide from 1×10^{-4} *M*. KCN to 5×10^{-4} *M*. KCN in no way altered the pattern of inhibition and only slightly increased the level of inhibition. After 3 days' incubation a 40 per cent. stimulation of O_2 uptake was occasioned by a concentration of cyanide which initially had caused an inhibition of almost 90 per cent. Subsequent to this the sensitivity of the disks to cyanide redeveloped.

A batch of disks taken from the same swede and maintained at 7° C. gave an essentially similar pattern in response to cyanide treatment at 25° C. but there were significant differences (Fig. 1B). The early decrease in sensitivity was much more gradual and the point of inflexion occurred a week later. In the light of the normal respiratory patterns for swede disks at different temperatures (for which see MacDonald and DeKock 1958*b*) this was not unexpected. The other difference was that although the initial level of inhibition was the same as that of disks at 25° C. this tissue always showed some cyanide sensitivity, the lowest value recorded being a 15 per cent. inhibition as compared with a 40 per cent. stimulation in the disks incubated at 25° C. The question thus arose was this consistently higher sensitivity a feature of disks exhibiting a lower metabolic rate, or was it the result of transferring disks washing at a low temperature to a higher temperature for respiratory measurements and thereby recording a temperature-induced respiration known to be highly cyanide sensitive (Commoner, 1940)? To test this possibility the cyanide sensitivity of a batch of disks maintained at 10° C. was measured at 10° C. and the results shown in Fig. 1C obtained. A stimulation of almost 40 per cent. was recorded after 7 days and the general pattern resembles that of disks pretreated and measured at 25° C. Clearly therefore, to obtain true values for the cyanide sensitivity of tissue, respiratory measurements should be carried out at the temperature of pretreatment.

(*b*) *Sugar-beet.* A typical cyanide inhibition curve for sugar-beet disks is that shown in Fig. 2A for disks pretreated at 15° C. and measured at 25° C. It differs from that obtained from swede disks in that after a substantial fall during the first day the level of inhibition gradually returned almost to the initial value before the sustained decline set in.

Fig. 2B illustrates the pattern obtained from disks pretreated and measured at 12° C. Due doubtless to the absence of a temperature-induced cyanide-sensitive respiration the general level of inhibition is lower than that in the preceding beet and the lowest point in the graph actually represents a 2 per cent. stimulation. The first fall-off in sensitivity is prolonged over 3 days but this is followed by what, as a result of experiments on several beet, has been established as the characteristic feature of sensitivity patterns in beet—a gradual return to a higher level of inhibition.

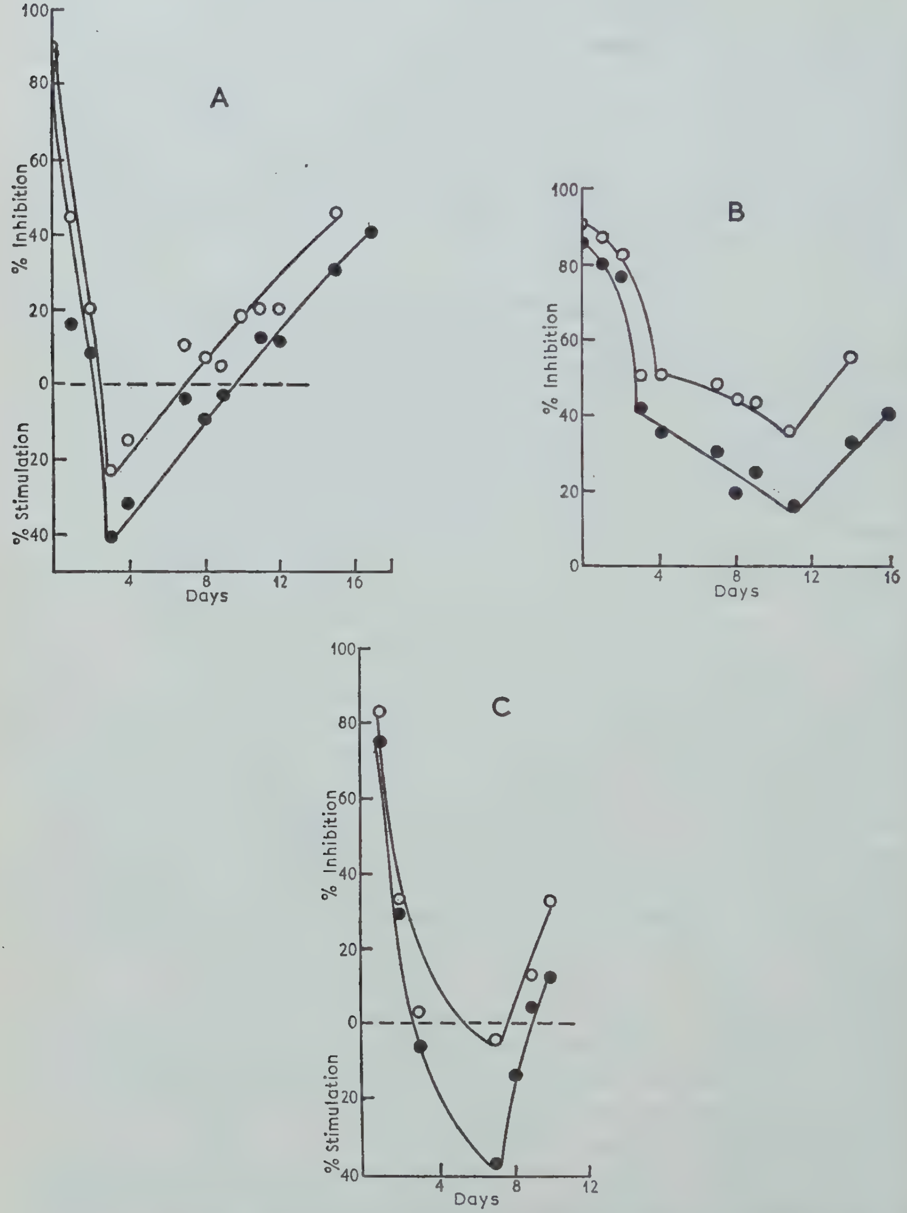

FIG. 1. Response of swede disks to cyanide expressed as a percentage of the oxygen uptake of the control. ○——○ Effect of 5×10^{-4} *M*. KCN, ●——● Effect of 1×10^{-4} *M*. KCN. Oxygen uptake measured at 25° C.: A—Disks pretreated at 25° C. B—Disks pretreated at 7° C. C—Oxygen uptake measured at 10° C., disks pretreated at 10° C.

A batch of disks from the same beet pretreated and measured at 25° C. yielded the values shown in Fig. 2C. Here the initial drop is not apparent but after a gradual increase in sensitivity for 6 days the level of inhibition falls away very rapidly, giving place to a slight stimulation before the trend is reversed as in swede disks.

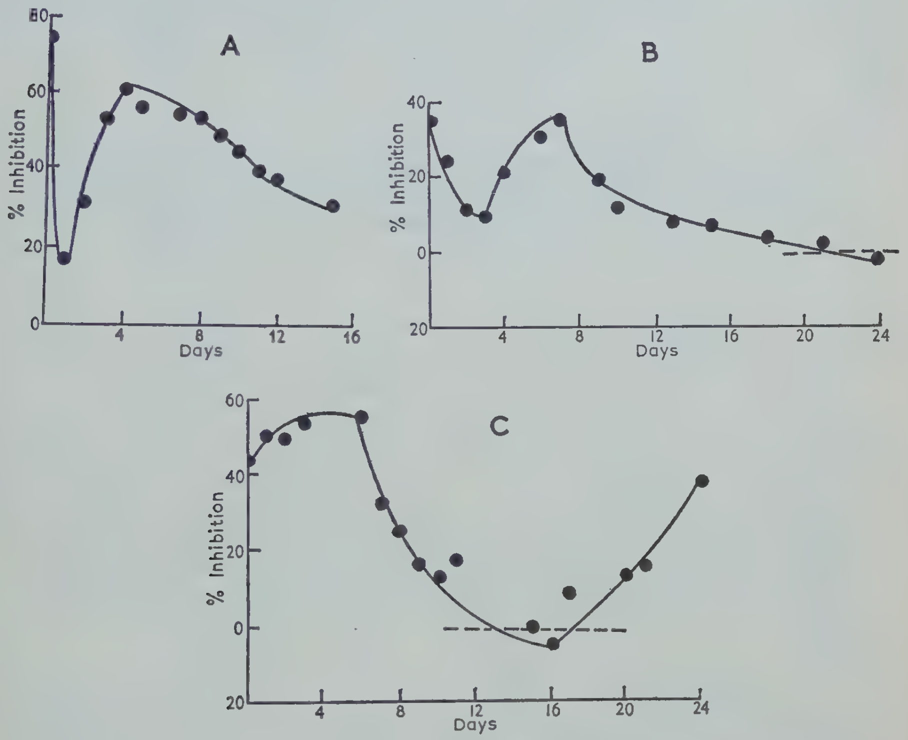

FIG. 2. Response of sugar-beet disks to 1×10^{-4} *M*. KCN expressed as a percentage of the oxygen uptake of the control: A—Oxygen uptake measured at 25° C., disks pretreated at 15° C. B—Oxygen uptake measured at 12° C., disks pretreated at 12° C. C—Oxygen uptake measured at 25° C., disks pretreated at 25° C.

(*c*) *Red beet*. Sensitivity patterns obtained from disks of red beet were very similar to that recorded for sugar-beet although the level of inhibition is somewhat lower. Disks pretreated and measured at 15° C. (Fig. 3A) show the initial drop followed by a well-marked period of increasing sensitivity typical of beet tissue. The subsequent decline continues, developing into a substantial stimulation before the final period of increasing sensitivity sets in.

As with sugar-beet, red-beet disks pretreated and measured at 25° C. (Fig. 3B) immediately show an increasing sensitivity. Here too the subsequent decline develops into a brief stimulation before the final return to inhibition.

(*d*) *Carrot*. A batch of carrot disks from several roots was randomized and divided into two lots which as before were pretreated and measured at 12° C. and 25° C.

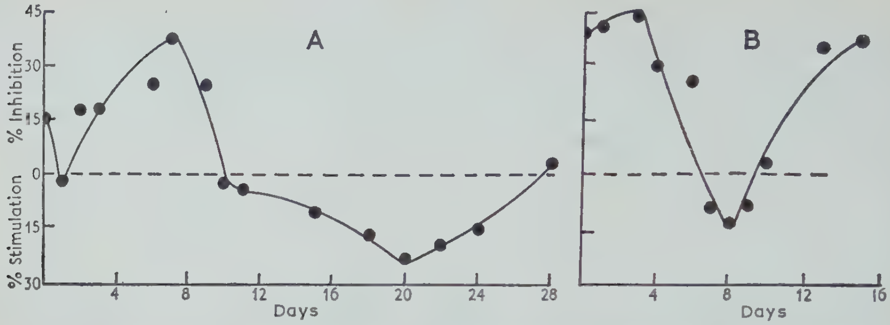

FIG. 3. Response of red-beet disks to 1×10^{-4} *M.* KCN expressed as a percentage of the oxygen uptake of the control: A—Oxygen uptake measured at 15° C., disks pretreated at 15° C. B—Oxygen uptake measured at 25° C., disks pretreated at 25° C.

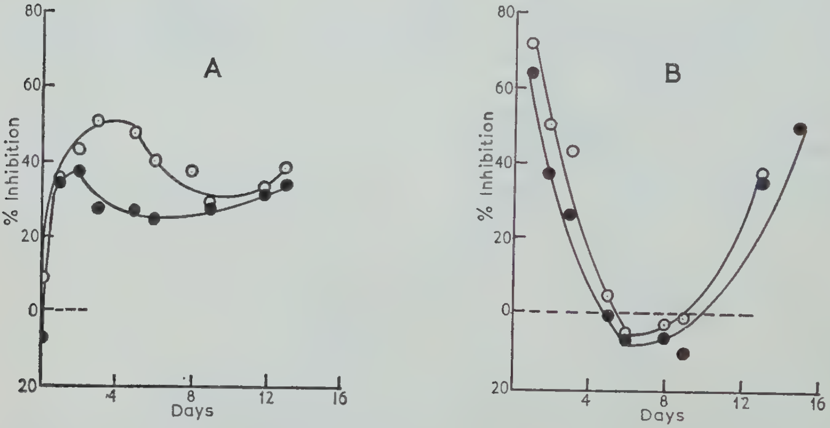

FIG. 4. Response of carrot disks to cyanide, expressed as a percentage of the oxygen uptake of the control: ●——● Effect of 1×10^{-4} *M.* KCN, ○——○ Effect of 5×10^{-4} *M.* KCN. A—Oxygen uptake measured at 12° C., disks pretreated at 12° C. B—Oxygen uptake measured at 25° C., disks pretreated at 25° C.

Fig. 4A illustrates the pattern of sensitivity to 1×10^{-4} *M.* KCN and 5×10^{-4} *M.* KCN exhibited by disks at 12° C. The freshly cut disks show very little sensitivity (a slight stimulation with 1×10^{-4} *M.* KCN and a slight inhibition with 5×10^{-4} *M.* KCN), but thereafter the level of inhibition gradually increases for 3 or 4 days. With 5×10^{-4} *M.* KCN this increase is followed by a decline which, however, is not so apparent with 1×10^{-4} *M.* KCN.

Unfortunately a value for freshly cut disks at 25° C. was not obtained, but on the basis of the value recorded for freshly cut disks at 12° C. (disks from the same batch of carrots) a fair assumption would be that the inhibition of disks at 25° C. would be low and considerable lower than that obtained from disks aged 24 hours. Fig. 4B shows the subsequent pattern. The sensitivity falls very rapidly, a slight stimulation being recorded before the reverse trend sets in. 5×10^{-4} *M.* KCN inhibited the oxygen uptake only slightly more than 1×10^{-4} *M.* KCN.

(*e*) *Potato.* The altered sensitivity of potato disks pretreated and measured at 25° C. (Fig. 5A) follows the same pattern as that of swede disks. After 48 hours a 70 per cent. inhibition had been replaced by a slight stimulation followed by a return to inhibition. The inhibition exerted by 5×10^{-4} *M.* KCN was more marked but the pattern was unaffected.

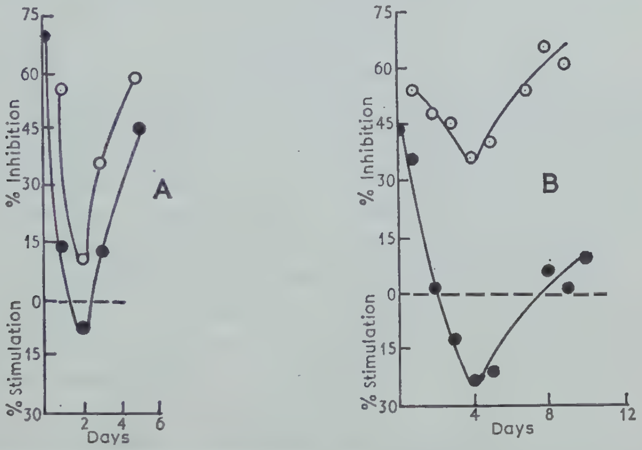

FIG. 5. Response of potato disks to cyanide, expressed as a percentage of the oxygen uptake of the control: ●——● Effect of 1×10^{-4} *M.* KCN, ○——⊙ Effect of 5×10^{-4} *M.* KCN. A—Oxygen uptake measured at 25° C., disks pretreated at 25° C. B—Oxygen uptake measured at 25° C., disks pretreated at 12° C.

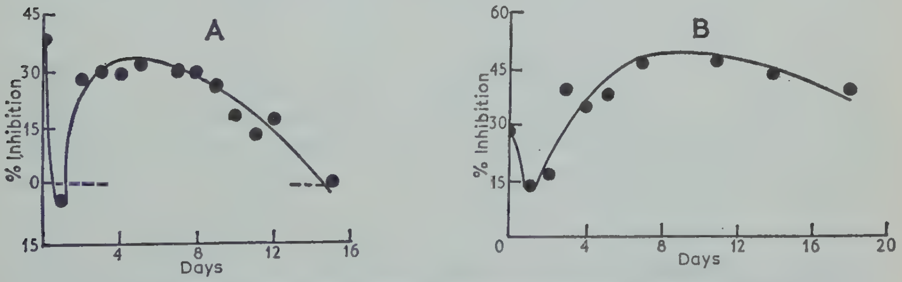

FIG. 6. Response of sugar-beet disks to treatment, expressed as a percentage of the oxygen uptake of the control: A—With $0{\cdot}5 \times 10^{-3}$ *M.* NaN_3; oxygen uptake measured at 25° C., disks pretreated at 15° C. B—With carbon monoxide ($CO:O_2 = 80:20$); oxygen uptake measured at 25° C., disks pretreated at 10° C.

Fig. 5B illustrates the pattern obtained from a different batch of potato disks pretreated at 12° C. These potatoes had been several months in storage. As one would expect from disks pretreated at a lower temperature the decline and subsequent increase is more protracted. The initial level of inhibition with 1×10^{-4} *M.* KCN was lower and gave way to a more pronounced stimulation. The inhibiting effects of 5×10^{-4} *M.* KCN were more severe.

2. *The effect of other heavy metal inhibitors on the respiration of sugar-beet disks*

The effect of carbon monoxide (80:20 $CO:O_2$) and $0{\cdot}5\times10^{-3}$ *M.* NaN_3 on the oxygen uptake of sugar-beet disks was investigated on two separate batches of disks. The pattern for azide (Fig. 6A) and the pattern for monoxide (Fig. 6B) are substantially similar to that shown for cyanide. Following an initial fall the sensitivity steadily increases for a few days before the final decline.

DISCUSSION

Wound Respiration

Wounding of plant tissue causes an immediate increase in respiration. This increased respiratory activity is extremely sensitive to cyanide (James, 1953*a*) and in sliced storage tissue, in which it is particularly noticeable, a several-fold increase may have occurred by the earliest time at which measurements are possible (Laties, 1957). It can be assumed therefore that the initial high sensitivity of disks to cyanide represents a wound respiration since uncut storage tissue is a mature tissue which would be relatively insensitive to cyanide. Although the respiration rate may continue to increase for several days this sustained increase is probably not a wound respiration *per se* but rather an induced respiration the development of which is associated with increased substrate availability (MacDonald and DeKock, 1958*b*). It may be inferred from the present study that the wound respiration as such is more transient, contributing a progressively lessening fraction of the total respiration, and its gradual extinction within a 48-hour period can probably be identified with the initial fall in cyanide sensitivity shown in Figs. 2A, 2B, and 3A.

The Effect of Cyanide on Growing Tissue

The cyanide-sensitivity values recorded for the five tissues investigated appear to fall into two distinct patterns. The pattern characteristic of swede and potato exhibits a continuous decline in sensitivity followed by a redevelopment of the inhibition. The beets, however, early in the life-span of the disks show a period of increasing sensitivity to cyanide (Figs. 2A, 2B, and 3A) which is here interpreted as testifying to a period of renewed growth. This increasing sensitivity (e.g. from 1 to 4 days in Fig. 2A) is to be distinguished from the increasing sensitivity phase (probably the result of cellular disorganization) noted in swede (e.g. Fig. 1A) the counterpart of which, in beet disks, occurs much later as shown in Figs. 2C, 3A, and 3B. The growth-phase period of increasing sensitivity in beet differs from the redevelopment of sensitivity shown in swede and potato (and latterly in beet itself) in that the former is generally accompanied by a temporary decrease in the level of cyanide-resistant respiration before this fraction reaches its maximum value. The commencement of the growth phase is obviously temperature-determined, being regarded in disks washing at a lower temperature (Fig. 2B), while in disks washing at 25° C. (Figs. 2C, and 3B) its early development obscures the decline in sensitivity due to the fall in wound respiration. This doubtless is partly responsible for the more modest increase in sensitivity of red-beet disks at 25° C. compared with those at 15° C. Carrot disks do not fit perfectly into either group, but the period of increasing sensitivity evident at 12° C. (Fig. 4A) suggests that their affinities lie with the beet group.

Swede and potato disks probably fail to exhibit the growth phase in their cyanide-sensitivity patterns because of the absence of cambial cells present in beet and carrot. Although parenchymatous cells are capable of renewed growth culminating in cell division (Sinnott and Bloch, 1941) growth in these disks, if occurring, must be insufficient to assert its influence on the cyanide-sensitivity pattern. Earlier evidence (MacDonald, 1955) based on anatomical investigation and fresh-weight increases had suggested that renewed growth leading to cell division occurred in sugar-beet disks and the cyanide-sensitivity patterns furnish corroborative evidence. High sensitivity to cyanide is an almost universal feature of embryonic, young, meristematic, and regenerated tissues, both plant (James, 1953*b*) and animal (Needham, 1942). The most striking exception to this general rule is that reported by Steward and Miller (1954) who found that explants of carrot tissue proliferating in a coconut-milk medium were relatively insensitive to cyanide, in comparison with relatively non-dividing tissue which was markedly cyanide sensitive. This latter tissue, however, showed a fresh-weight increase of the same order as that recorded in our disks.

The Effect of Cyanide on Ageing Tissue

A decrease in cyanide sensitivity is a characteristic feature of ageing tissue and the marked decline in sensitivity shown by the disks as they age is comparable with that manifested by ageing leaf tissue (MacDonald and DeKock, 1958*a*), although in potato and swede disks the decrease in wound respiration will contribute to the general fall in sensitivity. In beet tissue, however, due to the intervening growth phase, the decreases in sensitivity resulting from the extinction of wound respiration and from ageing processes are clearly segregated and it is evident that a very considerable decrease in sensitivity is associated with senescent changes in the disks. In all tissues investigated the rapidity of ageing and the decrease in cyanide sensitivity are temperature controlled. Thimann, Yocum, and Hackett (1954) also showed that potato disks aged at 25° C. lost their sensitivity to cyanide within 24 hours, whereas it took disks maintained in tap water 4 days to develop the same resistance.

Various explanations have been offered to account for the failure of cyanide to inhibit oxygen uptake in old tissue. Stenlid (1949), discussing the difference in sensitivity of young and old leaves to cyanide, azide, and dinitrophenol, suggested that the unresponsiveness of old tissue to these inhibitors was due to the unsuitability of the pH and to changes in permeability. Inhibition by azide is clearly more effective at a low pH, but with sugar-beet disks azide gave an inhibition pattern (Fig. 6A) essentially similar to that obtained with cyanide and carbon monoxide to neither of which do considerations of pH apply. We were not able to detect any differences in permeability to cyanide of disks of different ages, and the fact that as the disks age there is an increasing stimulation of respiration while in still older disks the inhibitory effect may reassert itself suggests that the cyanide molecules were present at the reactive sites. Lundegardh (1953) has shown that the cyanide effect on wheat roots is complete in about 1 minute. We found no evidence to suggest that the effect of the inhibitor on disks maintained in cyanide for several hours differed materially from that manifested at the outset.

A more likely explanation of the progressive decrease in cyanide inhibition is that some mechanism is operating to protect the respiratory enzymes. Cyanide itself can be inactivated by condensation with keto acids or sugar to form cyanohydrins and it has been shown (MacDonald and DeKock, 1958*b*) that in all storage tissues except swede there is a rapid increase in reducing sugar content with age. In common with the decrease in cyanide sensitivity the rate of increase in reducing sugar content is temperature-controlled. Furthermore the redevelopment of cyanide sensitivity generally occurs when the sugar content of the disks is nearing exhaustion. However, set against this must be the observation that the initial period of increasing sensitivity in beet disks occurs during the build-up in reducing sugar content. Nor does the failure to observe any decreasing effectiveness of cyanide in 6-hour experiments favour the theory of inactivation by cyanohydrin formation. Moreover the decreasing effectiveness of azide and carbon monoxide as inhibitors could not be accounted for by such a mechanism.

Recent work on auto-oxidizable *b* type cytochromes, which show no reaction with cyanide, isolated from both plant (Martin and Morton, 1955; Bendall and Hill, 1956; Yocum and Hackett, 1957; Hackett and Haas, 1958) and animal (Garfinkel, 1957) sources, has led to their being considered for the role of terminal oxidase in cyanide-resistant respiration (Hartree, 1957; Smith and Chance, 1958).

The Nature of the Respiratory Rise in Disks

The question inevitably arising from inhibitor studies in which a fraction of the oxygen uptake remains unaffected by the inhibitor is: can results so obtained be interpreted fairly as demonstrating qualitative differences in respiration? This question is of particular interest in disks the respiration of which is so very different quantitatively from that of uncut tissue.

Most investigators have accepted partial inhibition by heavy-metal inhibitors as evidence of collateral oxidase activity, but the validity of the assumption that the failure of cyanide or carbon monoxide to inhibit respiration either wholly or partly demonstrates the non-participation of cytochrome oxidase has not been allowed to go unchallenged (Hill and Hartree, 1953; Hartree, 1957; Smith and Chance, 1958). Anything less than 100 per cent. inhibition is not necessarily the result of dual oxidase activity since the percentage inhibition is determined by the mass action law. Moreover to obtain a level of inhibition even as 'complete' as that demanded by the dissociation constants of the reactant the oxidase itself must be operating near its maximum capacity. Otherwise if some such factor as the substrate supply or the activity of associated catalysts limited the respiratory level considerable inhibition of the unsaturated oxidase might occur without effect on the respiratory rate. However, since maximum inhibition of the disks was generally recorded shortly after slicing when the respiration rate was lowest, and since thereafter when both substrate availability and respiratory activity increased, the percentage inhibition decreased, the view that failure to record an inhibition was due to unsaturation of the oxidase becomes less probable. Nor does the relatively small increase in inhibition obtained with a fivefold increase in cyanide concentration suggest that a proportionate increase in inhibition has been exerted on a non-limiting step.

Since there is a progressive decline in the respiration rate of ageing tissue it has been suggested that electron transport is mediated by two oxidative pathways one of which, the cyanide-stable one, mediates the basal respiration which varies little over the life-span. The oxygen uptake additional to this basal level varies with age, being high in young tissue, and is mediated by a cyanide-sensitive oxidase. On this basis the increasing unresponsiveness to cyanide of ageing tissue is accounted for by postulating a decreasing activity of the sensitive system (Marsh and Goddard, 1939; Commoner, 1940; Robertson, Turner, and Wilkins, 1947; James and Beevers, 1950). Middleton (1955) further suggests that in beet disks the basal cyanide-resistant system is of limited activity and any respiratory increase induced by wounding, salts, &c., would be carried by the cyanide-sensitive system. The results obtained from ageing disks do not, however, allow any such clear-cut distinctions. There is no constancy in any fraction. During the development of respiratory activity there is at first an increase in both the cyanide-resistant and cyanide-sensitive fractions, although the latter decreases proportionately (see data in Appendix). In all tissues investigated the greater part of the respiratory increase was cyanide resistant. Thimann, Yocum, and Hackett (1954), Calo and Varner (1957), and Griffiths and Hackett (1957) also reported the cyanide-resistant increase in potato disks.

The inadequacy of all theories offered in explanation of the ineffectiveness (either wholly or partially) of cyanide as a respiratory inhibitor extends to, and in fact is emphasized by, the respiratory stimulations which may be induced by the inhibitor. Stimulations caused by cyanide and other inhibitors have been reported from both plant and animal tissues and recently have been discussed with reference to leaf tissue (MacDonald and DeKock, 1958*a*). It was shown that the cyanide sensitivity of leaf tissue was correlated with the iron status of the leaf. Iron deficient leaves are very sensitive to inhibitors, whereas iron toxic leaves are practically unaffected. However, the oxygen uptake of some toxic leaves was stimulated in the presence of heavy metal inhibitors, and since there is some evidence that in old tissues the high level of iron may exert an inhibitory effect on cellular respiration it was suggested that cyanide or carbon monoxide by complexing inhibitory heavy metals would permit a higher respiratory level. MacDonald and DeKock (1958*a*) further showed that if the inhibition values obtained from using various inhibitors at different concentrations on young tomato leaves are plotted against the inhibition values obtained from mature leaves, the points fall along a straight line. Consequently since tissues of varying age show a proportionate response to different inhibitors, irrespective of the specific effect of these inhibitors, it would seem that the tissue's response to the inhibitor is determined by particular equilibria within the cell.

It follows from this concept that the degree of inhibition or stimulation induced in any tissue will depend on the relative amounts of the reactants concerned (e.g. the ferric : ferrous balance) which in turn are determined by the metabolic status of the cell. Differences in the response of young and old, dormant or rejuvenated tissues to cyanide are therefore to be interpreted as reflecting changes in particular equilibria in the cell rather than the result of a switch-over from one type of oxidase to another. This is not to deny the possibility that more than one pathway for electron transport may operate in plant tissue, but it does seem as if the fractionation of respiration into basal and non-basal, and the allocation, on the basis of inhibitor studies, of these or other fractions to different oxidases is artificial.

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APPENDIX

(Tables 1-6)

TABLE 1

Oxygen uptake μl O_2/2 hr./10 disks

Swede disks (Fig. 1A)			
Days	Control	$+1\times10^{-4}$ *M.* KCN	$+5\times10^{-4}$ *M.* KCN
0	262	38	26
1	331	277	179
2	418	383	376
3	233	328	287
4	295	388	341
7	318	330	287
8	323	355	299
9	311	320	297
10	327	—	268
11	316	278	254
12	296	263	239
15	259	180	141
17	260	153	—

Swede disks (Fig. 1B)			
Days	Control	$+1\times10^{-4}$ *M.* KCN	$+5\times10^{-4}$ *M.* KCN
0	262	38	26
1	325	66	40
2	358	84	61
3	356	207	176
4	388	251	191
7	385	269	200
8	406	328	227
9	391	295	222
11	387	327	244
14	441	297	197
16	499	298	—

Swede disks (Fig. 1C)			
Days	Control	$+1\times10^{-4}$ *M.* KCN	$+5\times10^{-4}$ *M.* KCN
1	94	23	16
2	78	55	52
3	101	107	98
7	76	104	79
8	79	89	—
9	120	115	104
10	83	73	56

TABLE 2

Oxygen uptake μl O_2/2 hr./10 disks

Sugar-beet disks (Fig. 2A)		
Days	Control	$+1\times10^{-4}$ *M.* KCN
0	121	31
1	158	131
2	249	170
3	292	136
4	285	111
5	291	128
7	289	131
8	332	153
9	304	152
10	304	159
11	297	174
12	307	189
15	270	180

Sugar-beet disks (Fig. 2B)		
Days	Control	$+1\times10^{-4}$ *M.* KCN
0	31	20
1	49	37
2	78	69
3	91	82
4	94	74
6	109	76
7	127	83
9	143	115
10	132	119
13	146	135
15	145	135
18	134	129
21	153	150
24	148	150

Sugar-beet disks (Fig. 2C)		
Days	Control	$+1\times10^{-4}$ *M.* KCN
0	118	66
1	243	120
2	303	153
3	383	175
6	331	149
7	254	172
8	273	204
9	255	215
10	232	202
11	235	195
15	196	198
16	166	175
17	198	182
20	152	133
21	134	113
24	139	86

TABLE 3

Oxygen uptake $\mu l\ O_2/2$ *hr./10 disks*

Red-beet disks (Fig. 3A)

Days	Control	$+1\times10^{-4}$ *M.* KCN
0	27	23
1	53	54
2	100	82
3	111	91
6	155	116
7	172	108
9	137	104
10	119	122
11	127	132
15	101	111
18	79	92
20	69	85
22	64	77
24	59	68
28	36	35

Red-beet disks (Fig. 3B)

Days	Control	$+1\times10^{-4}$ *M.* KCN
0	117	71
1	322	188
3	399	226
4	305	216
6	285	211
7	210	229
8	202	227
9	170	184
10	171	165
13	167	108
15	154	98

TABLE 4

Oxygen uptake $\mu l\ O_2/2$ *hr./10 disks*

Carrot disks (Fig. 4A)

Days	Control	$+1\times10^{-4}$ *M.* KCN	$+5\times10^{-4}$ *M.* KCN
0	82	88	75
1	141	93	92
2	135	85	77
3	121	88	60
5	149	108	78
6	147	110	88
8	142	—	89
9	125	90	89
12	118	81	79
13	107	70	66

Carrot disks (Fig. 4B)

Days	Control	$+1\times10^{-4}$ *M.* KCN	$+5\times10^{-4}$ *M.* KCN
1	448	156	126
2	381	241	189
3	380	275	212
5	237	236	224
6	240	252	255
8	236	248	240
9	191	209	192
13	138	86	88
15	146	74	—

TABLE 5

Oxygen uptake $\mu l\ O_2/2$ *hr./10 disks*

Potato disks (Fig. 5A)

Days	Control	$+1\times10^{-4}$ *M.* KCN	$+5\times10^{-4}$ *M.* KCN
0	30	9	—
1	165	141	73
2	162	173	145
3	230	201	147
5	210	113	86

Potato disks (Fig. 5B)

Days	Control	$+1\times10^{-4}$ *M.* KCN	$+5\times10^{-4}$ *M.* KCN
0	84	47	—
1	171	109	79
2	187	184	97
3	196	220	107
4	175	216	111
5	207	249	123
7	218	—	100
8	267	253	93
9	276	271	108
10	311	282	—

TABLE 6

Oxygen uptake μl O_2*/2 hr./10 disks*

Sugar-beet disks (Fig. 6A)

Days	Control	$0{\cdot}5 \times 10^{-3}$ M. NaN_3
0	121	74
1	158	165
2	249	175
3	292	204
4	285	202
5	291	198
7	289	199
8	332	230
9	304	221
10	304	247
11	297	257
12	307	268
15	270	268

Sugar-beet disks (Fig. 6B)

Days	Control	CO.
0	105	75
1	98	83
2	129	106
3	177	107
4	178	116
5	190	117
7	197	105
11	199	106
14	238	134
18	282	169

An Electron Microscope Investigation into the Structure of the Floridean Pit

BY

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With two Plates

ABSTRACT

Ultra-thin sections of the red algae *Rhodymenia palmata* and *Laurencia caespitosa* were examined in the electron microscope in order to determine the fine structure of the Floridean pits. The pits found in the two species examined are shown to be quite different from each other, in *Rhodymenia* the pit is open so that the cytoplasm is continuous through the cell wall, whilst in *Laurencia* the pit is completely closed by a lamella of the cell wall. These two types are shown to be equivalent to those described by Jungers (1933) on the basis of light microscope observations.

THE Floridean pit is a characteristic feature of the cell walls of the subclass of red algae known as the Florideae. The absolute structure and function of this pit have been the subjects of much controversy, due in the main to the limited resolving power of the light microscope, so that even the presence or absence of a pit-closing membrane is a doubtful feature. Here then is a problem to which the application of the electron microscopy of ultra-thin sections could be expected to be of considerable interest.

The light microscopy of the Floridean pit is ably reviewed by Kylin (1940), and the following brief outline of previous work is based upon this review. An early description of the Floridean pit is that of Schmitz (1883) who stated that the pit is closed by a membrane in close contact with which are two plates, lying one on either side. The plates were found to stain with haematoxylin and, therefore, were described as protoplasmic in nature. Schmitz also saw plasmodesmata passing through the pit membrane, and although Falkenburg (1901), who held the same opinions, could not demonstrate the existences of plasmodesmata, he stressed that they must exist. Kienitz-Gerloff (1902) and Kohl (1902) also reported that plasmodesmata are not visible in the pit-closing membrane. In contrast to these views, Mangenot (1924) described the pit found in *Griffithsia* as having no membrane, so that the protoplasts of adjoining cells were considered to come into direct contact. Miranda (1930) returned to the older idea of the presence of a membrane and in *Bornetia secundiflora* he detected the existence of connecting protoplasmic strands. The widely varying structures ascribed to Floridean pits by critical observers immediately suggest that they were describing different objects,

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and this is made more likely by the fact that they were often examining different species of red algae. It is not surprising, therefore, that Jungers (1933) decided that two kinds of pit occur in the Florideae, named by him the *Polysiphonia* type and the *Griffithsia* type. The *Polysiphonia* type was described as two densely staining plates separated by a membrane with no plasmodesmata, and the *Griffithsia* type as a dense, biconvex lens-shaped body without a closing membrane. Müldorf (1937) questioned the proposed structure of the *Polysiphonia* type of pit and suggested that the bodies on either side of the membrane are not plates but in fact rings which are part of the cell wall. The membrane he regarded as double, representing the ectoplasmic membranes of the two protoplasts concerned. Kylin (1940) examined *Bonnemaisonia asparagoides* in the hope of clarifying the situation. He first observed unstained material and saw the two refractive bands first described by Schmitz, and between them he saw a membrane. When the pit was inclined, however, he was able to distinguish that the 'plates' were in fact rings just as Müldorf had described. By staining reactions Kylin concluded that the refractive rings are part of the wall, yet do not contain cellulose.

The purpose of the present paper is to describe the results of an investigation into the structure of the Floridean pit as revealed by the electron microscope. Use has been made of ultra-thin sectioning in the examination of two species of red algae, *Rhodymenia palmata* and *Laurencia caespitosa*, which were selected because in a complex multicellular thallus any section is likely to pass through many pits.

Materials and Methods

The algae for examination were collected at Cullercoates on the north-east coast of England and were transported in polythene bags to the laboratories at Leeds and fixed in 2 per cent. osmium tetroxide buffered to pH 7·3 in a veronal-acetate buffer. After washing in diluted buffer solution the small pieces of alga were taken quickly through the alcohols and finally to a mixture of butyl and methyl methacrylates in the ratio 3:1, containing 2 per cent. Luperco. This was polymerized in small gelatine capsules at a temperature of 45–48° C. The algal material was suitably oriented in partially polymerized methacrylate. Fully polymerized blocks were cut in the usual way on a Hodge type microtome (Hodge, Huxley, and Spiro 1954) constructed in this laboratory. The methacrylate was removed from the sections whilst mounted on the formvar films of the electron microscope specimen grids, and the grids were then shadowed with Pd-Au.

Results

(*a*) *Rhodymenia palmata*

A low-power electron micrograph of part of a transverse section of *Rhodymenia palmata* is represented in Plate 1, Fig. 1. A pit can be seen situated in the top centre of this micrograph, interrupting the wall which lies parallel to the long axis of the plate. The pit, which is very electron dense, appears to be about 1·3 μ in diameter, although it is unlikely that the section is strictly median so that the pit may be much larger. The cell wall adjacent to the pit aperture can be seen to be much narrower than elsewhere. In this micrograph it is impossible to make out any details of the dense 'plug' situated in the pore. In Plate 1, Fig. 2 the plane of the section has passed close to a pit without actually including it, and the close proximity of the pit can be detected by the very pronounced 'waisting' of the cell wall which can be seen here. The middle lamella, represented here as a fine electron-dense line is particularly prominent in the region of wall near the pit, and the remainder of the cell wall appears to be uniformly amorphous. The non-appearance of microfibrils is to be associated with the very low proportion of cellulose present in the walls of *Rhodymenia* (Naylor and Russell-Wells, 1934; Cronshaw, Myers, and Preston, 1958). In Plate 2, Fig. 3 the plane of the section has passed through the aperture of the pit, which is again in the order of 1 μ diameter. The pit opening is quite clearly filled with protoplasm which is apparently continuous from cell to cell. It is possible that in the specimen preparation a fine cytoplasmic membrane has been lost, but it is quite certain that the cell wall at least is completely interrupted by the pit. In this section the middle lamella may be seen to fork close to the pit.

(*b*) *Laurencia caespitosa*

In Plate 2, Fig. 4 is illustrated a micrograph of a section of *Laurencia caespitosa* in which a cell wall (running parallel to the long axis of the page) is interrupted by a pit. The pit is seen to be about 3·3 μ diameter and is therefore two to three times as large as the *Rhodymenia* pit. A body which is very electron dense and continuous with the middle lamella of the cell wall completely seals the pit. In contrast to *Rhodymenia* the cell wall is cut off very abruptly so that the gradual 'waisting' is not seen. A similar pit is illustrated in Plate 2, Fig. 5, and here the pit-closing lamella can be more clearly distinguished from the cytoplasm. This closing lamella is about 0·5 μ in thickness at its widest point and becomes slightly narrower at the edges where it can be seen to be embedded into the cell wall. In no section were plasmodesmata detected crossing the pit-closing membrane.

Discussion

The two species of red algae examined in this investigation were treated in exactly the same way, and appeared to be in similar stages of development, yet they show striking differences in pit structure. In *Rhodymenia* the pit appears to be completely open so that the wall is interrupted by a circular aperture through which the cytoplasm of adjacent cells is continuous. In some cases the cytoplasm appears to be concentrated in the pit to form a dense plug. By contrast, the pit found in *Laurencia caespitosa* is closed by a cell-wall membrane. An additional difference is that in *Rhodymenia* the cell wall becomes gradually narrower around the pit, whilst in *Laurencia* the wall other than the pit-closing membrane comes to an abrupt end.

It is interesting to compare these results with those obtained by workers using the light microscope. Early workers assumed that pit structures were uniform throughout the Florideae and attempted to compare the results obtained using different species, and although many of their observations were correct it was left to Jungers (1933) to postulate that two kinds of pit are in existence. Moreover Jungers described all the essential features of the two types. In the *Polysiphonia* type he detected a closing membrane with no plasmodesmata, and therefore *Laurencia* belongs to this group. The *Griffithsia* type pit he described as open, but with the aperture occupied by a dense biconvex lens-shaped body. This description accurately fits the *Rhodymenia* pit.

In future work with the electron microscope it would be profitable to explore other species in the Florideae in order to determine the distribution within the group of the two types of pit, and it is not unlikely that further variation of structure will be found. It is also possible that an electron microscope investigation of the origin and development of the Floridean pit would lead to an identification of the functions of the pit.

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EXPLANATION OF PLATES

FIG. 1. Electron micrograph of a transverse section of *Rhodymenia palmata* (Magn. × 9,000). Methacrylate removed, shadowed Pd-Au. Wall, W; cytoplasm, C; pit, P.

FIG. 2. Electron micrograph of a transverse section of *Rhodymenia palmata* (Magn. × 15,000). Methacrylate removed, shadowed Pd-Au. Lettering as in Fig. 1.

FIG. 3. Electron micrograph of a transverse section of *Rhodymenia palmata* (Magn. × 22,500). Methacrylate removed, shadowed Pd-Au.

FIG. 4. Electron micrograph of a section of *Laurencia caespitosa* (Magn. × 18,000). Methacrylate removed, shadowed Pd-Au.

FIG. 5. Electron micrograph of a section of *Laurencia caespitosa* (Magn. × 15,000). Methacrylate removed, shadowed Pd-Au.

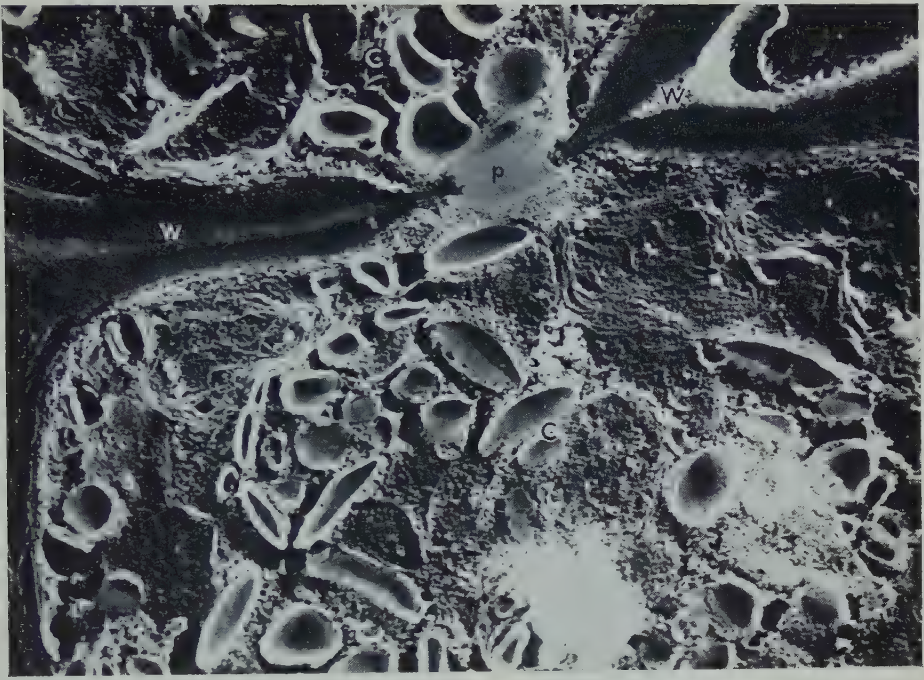

FIG. 1

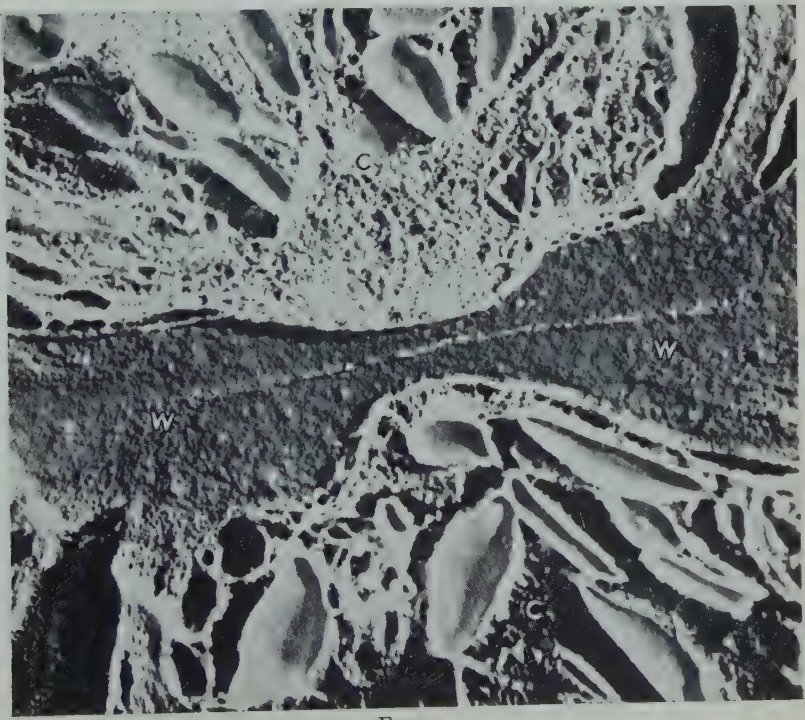

FIG. 2

A. MYERS, R. D. PRESTON, and G. W. RIPLEY

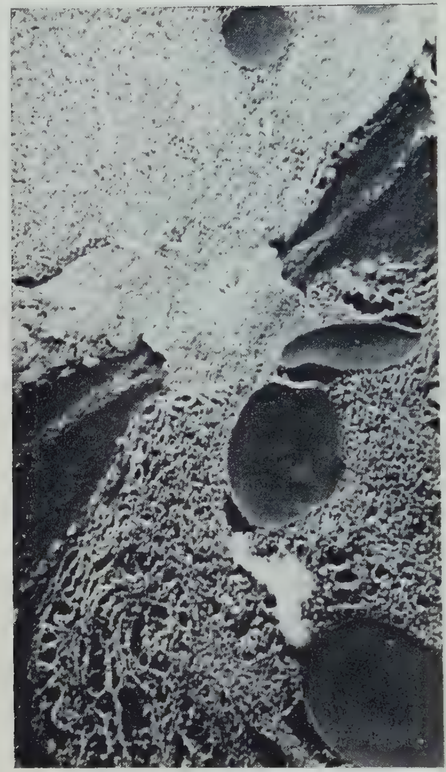

FIG. 3

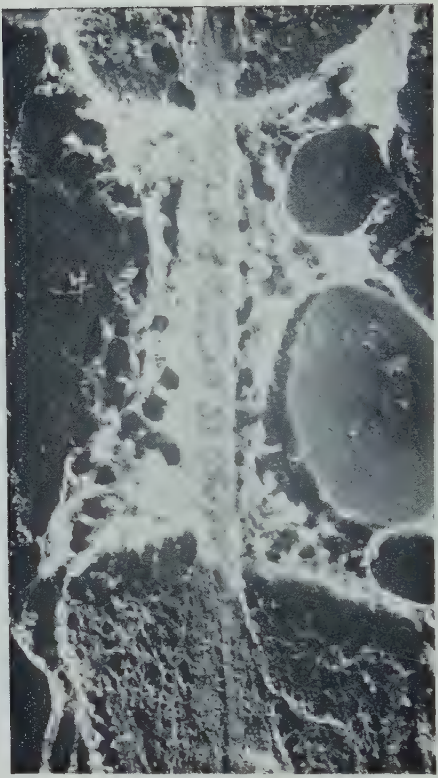

FIG. 4

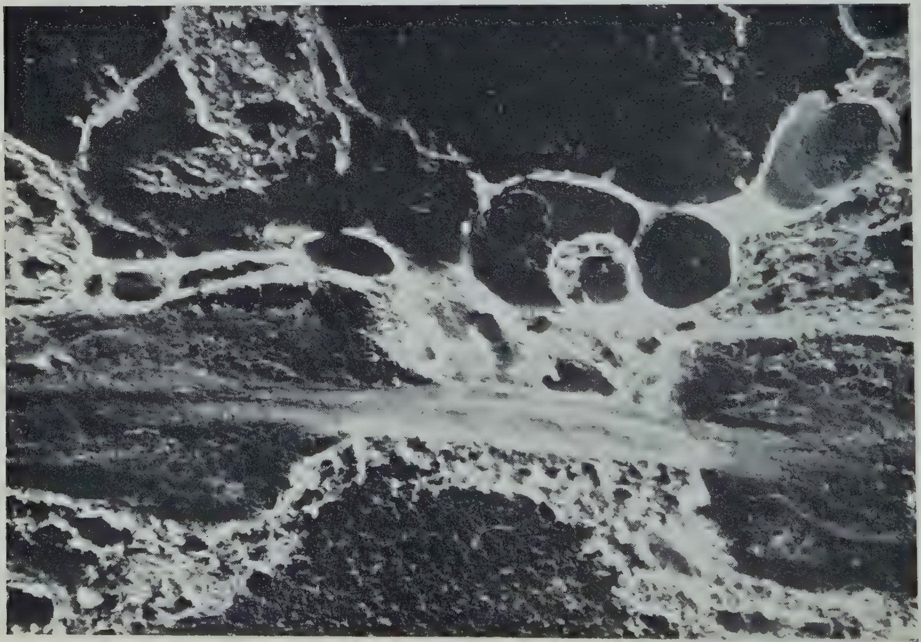

FIG. 5

A. MYERS, R. D. PRESTON, and G. W. RIPLEY

The Oxygen Factor in the Development and Function of the Root Nodules of Alder

BY

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With one Plate and one Figure in the text

ABSTRACT

The effect of the supply of oxygen to the root systems of alder plants growing in water culture has been studied in experiments in which oxygen–nitrogen gas mixtures were bubbled through the culture vessels. When the root systems were inoculated with the nodule organism in the presence of different levels of oxygen it was found that the number of nodules was progressively reduced as the oxygen level was lowered from the normal 21 per cent.

In a second experiment the effect of the variation of oxygen supply on the growth of already nodulated plants and of non-nodulated plants, the latter supplied with combined nitrogen, was compared. The nodulated plants proved to be considerably more sensitive to oxygen reduction. It is concluded that oxygen supply is of special importance in the development and function of the alder nodule and the possible reasons for this are discussed.

INTRODUCTION

THE work reported in this paper forms part of an investigation of the oxygen relations of alder nodules which has been made in the Botany Department, University of Glasgow, and which has involved short-term tests of fixation of isotopic nitrogen by detached alder nodules under different oxygen tensions, studies of nodule respiration, and long-term culture of nodulated plants maintained at different oxygen levels. Preliminary results from the first two approaches were given by Bond and MacConnell (1955). The third approach is the subject of the present paper.

It is frequently suggested on thermodynamic grounds that an early formation of oxygen derivatives of nitrogen during fixation is probable, but little critical evidence is available on the effect of the oxygen supply on the fixation process. The root nodules of alder are of special interest in this connexion for they have nitrogen-fixing properties and there is evidence from field observations which suggests that oxygen may be of special significance in the development and function of alder nodules. A massive development of nodules is often to be found just below the soil surface. In his ecological studies of alder, McVean (1956) drew a distinction between deep-growing anchoring roots and nutritional roots growing near the soil surface and bearing most of the nodules. He also observed that while nodules were present on trees growing in completely water-logged soil, they were much sparser than under conditions of better aeration.

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This localization of the nodules might be because the nodule organism, which is possibly highly aerobic, is limited to the upper layers of the soil. But good aeration might also be needed for the actual infection of the roots and for vigorous development of nodules. Such developments are bound up with metabolism, and a curtailment of respiration or of fixation within growing nodules because of oxygen deficiency would be likely to reduce growth-rates in the nodules concerned.

The present experiments were planned to throw light on these matters. The investigation was carried out in two stages, in order to separate oxygen effects on nodule initiation and those on later stages in nodule development when fixation of nitrogen becomes active. In the first stage, young plants whose root systems were maintained at different oxygen levels were inoculated with the nodule organism, and the number of nodules initiated determined after a short growing period. In the second stage already nodulated plants, previously grown under uniform conditions, were subject to different levels of oxygen supply and the effect on their subsequent growth and fixation of nitrogen studied. Also in this second stage, a parallel experiment with non-nodulated plants supplied with combined nitrogen was included in order to aid in the detection of specific effects of oxygen on nodule growth and function.

METHODS

The principle of the method employed in the experiments is that the plants were grown in culture solution in such a way that their root systems were sealed off from the natural atmosphere and exposed to gas mixtures of varied composition while the shoots were growing in normal greenhouse conditions.

In the case of the nodule initiation experiment the plant containers used were wide glass jars (8 × 13 cm.) holding 400 ml. of culture solution and fitted with waxed cork stoppers into which five seedlings could be inserted and sealed with 'plasticine' around the hypocotyl. Small uniform bubbles could be released at the foot of each jar through the obliquely ground end of a length of very fine capillary attached to a gas inlet tube. Another short length of capillary tube provided a gas outlet.

In the experiment on the further growth of already nodulated plants, the plants were set up individually in large test-tubes (20 × 3·5 cm.), each of which held approximately 200 ml. of culture solution. The tubes were closed with three-holed rubber stoppers, one hole being for the plant, a second for the gas exit tube, and the third for the gas inlet tube. The latter led to a cubical earthenware diffuser block at the foot of the test-tube. The tubes were placed in racks of six and attached to a gas manifold. Similar tubes were used by Ferguson and Bond (1954) for their root enclosure experiments on red clover.

Cylinders containing oxygen–nitrogen gas mixtures were obtained from British Oxygen Gases, Ltd. The precise oxygen levels, ranging from 21 to 1 per cent., in the cylinder gases were checked by means of the Haldane gas analysis apparatus. The flow of gas into the culture vessels was suitably adjusted to keep the culture solution at the appropriate equilibrium value as regards dissolved oxygen content. The latter was determined by the Winkler process, using the 1 ml. syringe pipette described by Fox and Wingfield (1938).

The plants used were raised from locally collected seed of alder (*Alnus glutinosa* (L.) Gaertn.) sown in trays of sand. On attaining the two-leaf stage the seedlings were transferred to water culture in Crone's culture solution free of combined nitrogen. Where nodulated plants were required an inoculum was prepared by grinding 2 g. of nodules from other alder plants in 10 ml. of distilled water. This extract was brushed on to the root systems of the plants.

The culture solution was maintained at pH 6·3 during the experiments (except in one case to be detailed later) and solution changes were made at three-weekly intervals. The complications which would have arisen in the gas-supply lines prevented the randomization of jars and tubes, but a fortnightly change was made in the relative position of each group of plants on the greenhouse bench.

At the close of the nodule initiation experiment, counts were made of the actual number of infection points (that is, the points on the root system actually invaded by the nodule organism and from which branched nodules would eventually develop as the plant matured) and of the number of nodule lobes already formed at each. In the experiment on the further growth of already nodulated plants dry weights of individual plants were obtained after drying them overnight in an oven at 95° C. and nitrogen estimations were made by Kjeldahl analysis.

DATA OBTAINED

Nodule initiation experiment

In this experiment the alder seedlings were transferred from sand directly into the gas-flow containers, four jars being set up at each of four oxygen levels, viz., 21, 12, 5, and 1 per cent., and 2 days later were inoculated.

At the higher oxygen levels, nodule initials marked as red swellings on the roots were first noted 10 days after inoculation. Some further days elapsed before nodules were detectable at the 1 per cent. oxygen level and in these plants the nodules were white in colour and thus free of anthocyanin.

When the plants were harvested, some 5 weeks after inoculation, all were at the four-leaf stage with shoots 1–2 cm. in height. Although there were notable differences in the nodule development at the different oxygen levels, the commencement of fixation was too recent for these to be reflected in the growth of the plants.

From Table 1 it will be seen that each lowering of the oxygen level is accompanied by a significant reduction in the number of infection points. The mean number of lobes per nodule cluster remains at approximately 1·5. The nodules were too immature to be detached and their weight determined, but there is no doubt that the amount of nodule tissue per plant fell with diminishing oxygen in much the same proportion as the number of infection points.

The experiment thus demonstrates that curtailment of oxygen supply severely reduces the extent of infection of the roots by the nodule organism (or at any rate, infection followed by nodule initiation), so that under such conditions the plant is poorly nodulated.

TABLE I

Effect of the Oxygen Supply to the Root System on Nodule Initiation in Alder

Percentage oxygen supplied	Mean no. per plant		Relative values	
	Infection points*	Nodule lobes	Infection points	Nodule lobes
21	10·6	16·7	100	100
12	7·9	11·2	75	67
5	5·2	8·5	49	51
1	2·2	3·6	21	21

* The difference between the number of infection points required for significance ($P = 0{\cdot}05$) calculated from an analysis of variance is 1·5.

The effect of oxygen supply on the further growth of already nodulated alder with a parallel study of non-nodulated plants.

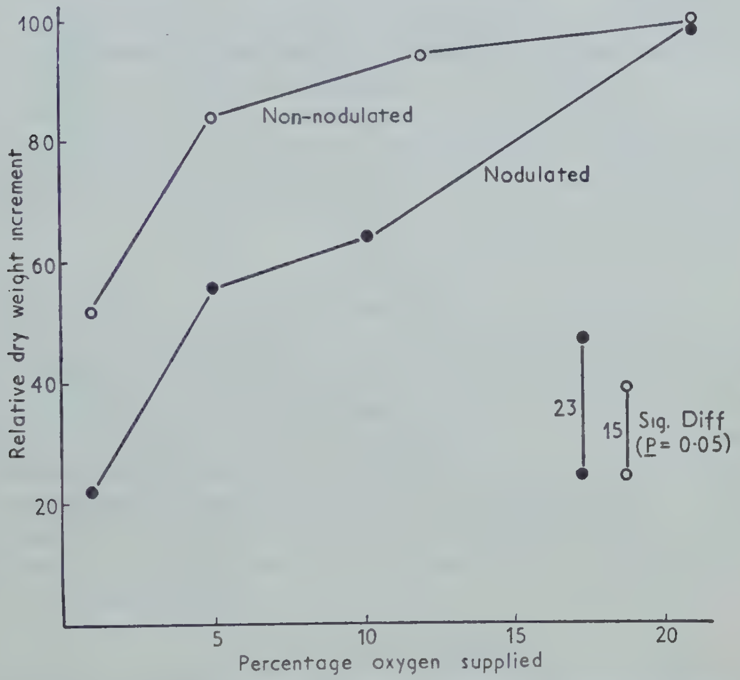
Due to restrictions on the availability of gas cylinders the two parts of this experiment were carried out in successive years during the same seasonal period (May to August), nodulated plants being grown in the first year and non-nodulated plants supplied with combined nitrogen in the second.

(*a*) *Nodulated plants*: Alder seedlings were inoculated 2 days after transfer into nitrogen-free Crone's solution in temporary containers and the first nodules appeared 2 to 3 weeks later. Four weeks after transplanting, the pH of the culture solution was lowered from its initial value of 6·3 to 5·5, this being the first step in lowering the pH to the range found by Ferguson and Bond (1953) to be the most suitable for alder growth subsequent to nodulation. At this stage also a small amount of combined ammonium nitrogen was supplied to each jar at the rate of 2 mg. per litre. This was a temporary measure to sustain the plants until fixation became really active, and the solution was changed back to the nitrogen-free form 2 weeks later. The pH was then lowered to 5·0, the value maintained throughout the remainder of the experiment.

When the plants were approximately 2 cm. in shoot height, bore 4–5 true leaves, and had several small clusters of root nodules present they were transferred to the gas-flow culture tubes. Eighteen plants were set up at each of four oxygen levels, viz., 21, 10, 5, and 1 per cent. A harvest of twelve such plants showed them to have a mean dry weight of 35 mg. and a mean total nitrogen content of 0·44 mg. These values were later used to calculate growth increments during the experiment.

A difference in growth between plants at different oxygen levels became noticeable after 2 weeks of gas flow. Plants at the lower oxygen levels tended to lag behind in growth and to show yellowing of the leaves. At harvest, after 8 weeks of gas-flow culture, there were pronounced differences in size especially between the plants at the two extreme oxygen levels. Typical plants at harvest are shown in the plate, Fig. 1.

The curtailment of growth with fall in oxygen level is clearly demonstrated by relative dry-weight values in Text-fig. 1. The effect is confirmed by the nitrogen content of the plants, the mean increases in total nitrogen being 8·8, 5·6, 4·7, and 1·7 mg. at the 21, 10, 5, and 1 per cent. oxygen levels respectively. Furthermore, the mean percentage nitrogen contents of the dry matter of the plants in order of decreasing oxygen supply were 2·53, 2·41, 2·34, and 1·83, values consistent with the visual evidence of nitrogen deficiency at the lower oxygen levels. Mean increases in nodule dry weight during the period were 20, 11, 9, and 4 mg. per plant respectively.



TEXT-FIG. 1. A comparison of the dry-weight increments shown by nodulated and non-nodulated alder plants, the latter supplied with ample combined nitrogen, grown with their root systems provided with different oxygen supply. The increments shown at 21 per cent. oxygen are taken as 100.

(*b*) *Non-nodulated plants*: The same general technique as used with the nodulated plants was followed except that inoculation was omitted. Precautions were taken to avoid infection by the nodule organism and no plants became inadvertently nodulated. The plants were grown on in temporary containers with 50 mg. nitrogen supplied as ammonium sulphate until they had attained a stature similar to that of the nodulated plants at the start of that part of the experiment. Fourteen such plants were set up at each of four oxygen levels (21, 12, 5, and 1 per cent.) in the gas-flow tubes with the culture solution again containing 50 mg. ammonium nitrogen per litre. The nitrogen level in the culture solution was raised to 100 mg. per litre in the fourth week of gas flow to ensure that growth was not retarded in any way by shortage of nitrogen. Plant growth in this experiment was very rapid and the gas-flow culture was maintained for only 5 weeks, by which time the plants were comparable in development to the nodulated plants at the stage when the latter were harvested.

Typical plants at harvest are shown in the plate, Fig. 2, and the similarity of development at the three higher oxygen levels may be noted, a feature shown also in Text-fig. 1. Dry-weight increments remained statistically unaffected by oxygen reduction until the final drop from 5 to 1 per cent. These plants thus display a markedly lower sensitiveness to curtailment of oxygen supply than do nodulated plants, the effect only becoming detectable at the lowest oxygen levels.

DISCUSSION

It has been shown in the nodule initiation experiment that a high oxygen level around the alder root system favours infection by the nodule organism and thus leads to more abundant nodulation. Existing knowledge of the infection process in non-legumes is too meagre to permit of much further analysis of this finding. There is evidence that inoculation is followed by a root hair deformation as in legumes (Fletcher, 1955; Pommer, 1956), but the actual method of penetration has not been satisfactorily demonstrated. Presumably this infection is preceded by a multiplication of the nodule organism in the rhizosphere under the influence of specific root secretions, possibly with a production by the organism of special infective stages not present in the crushed nodule inoculum. In the present experiment the curtailment of oxygen supply might have restricted the growth of the nodule organism either outside or inside the root, assuming the nodule organism to be highly aerobic. It is unlikely that the critical effect of reduced oxygen supply was on root hair development since the nodules tended to form the roots existing prior to inoculation and to the establishment of differential oxygen treatment. The marked curtailment of nodule formation under nearly anaerobic conditions indicates that the process is much more sensitive to oxygen scarcity than the legumes examined by Ferguson and Bond (1954). Furthermore, it is probable that the beneficial effect of forced aeration on the nodulation of alder plants observed by Ferguson and Bond (1953), and ascribed by them to a stirring effect, was in fact due to improved oxygen supply.

Considering now the results of the experiments relating to the effect of oxygen supply on the development of already nodulated plants, it has been shown that nodulated plants, dependent on nodule nitrogen for their growth, are much more sensitive to reduction of oxygen concentration than are non-nodulated plants supplied with combined nitrogen in amounts ample for growth. The reduced growth eventually shown (below 5 per cent. oxygen) by the non-nodulated plants is no doubt due to interference with the respiratory activities of the roots which in turn could curtail salt uptake. With 5 per cent. or more of oxygen present these effects were insufficiently exerted for a significant change in growth to be produced. Since there is no reason to suppose that the roots of nodulated plants differ materially in their oxygen requirements from those of non-nodulated plants, then it follows that the greater sensitiveness of nodulated plants to reduced oxygen supply is due to the relatively high oxygen requirement of the nodules. That the nodules, at reduced oxygen tension, were giving inadequate metabolic service to the plants is indicated by the visual and analytical evidence of nitrogen deficiency in these plants, already noted.

The further analysis of the results is complicated by the fact that both formative and functional effects may be involved in this long-term experiment. The effect might be simply that the further growth of the nodules present at the commencement of gas flow could not take place adequately under conditions of reduced oxygen supply, so that the physical basis for fixation was not provided. However, evidence has been provided in short-term tests by Bond and MacConnell (1955) that fixation by detached alder nodules is markedly sensitive to oxygen supply and it is reasonable to assume that a similar impairment of function occurred in the attached nodules in the present experiments. If the first-formed nodules were thus unable to fix nitrogen at the necessary rate, then the further growth of the plant and of the nodules would suffer.

The foregoing experiments show, however, that the oxygen factor is of considerable importance in the initiation and subsequent development and function of alder nodules. It may be concluded that nodulation in alder will be restricted in any environment where the oxygen concentration is substantially lower than that in air.

ACKNOWLEDGEMENTS

The author is indebted to Dr. G. Bond for his guidance throughout this investigation, part of which was carried out during the tenure of a Carnegie Research Scholarship. Photographic assistance was provided by Mr. W. Anderson.

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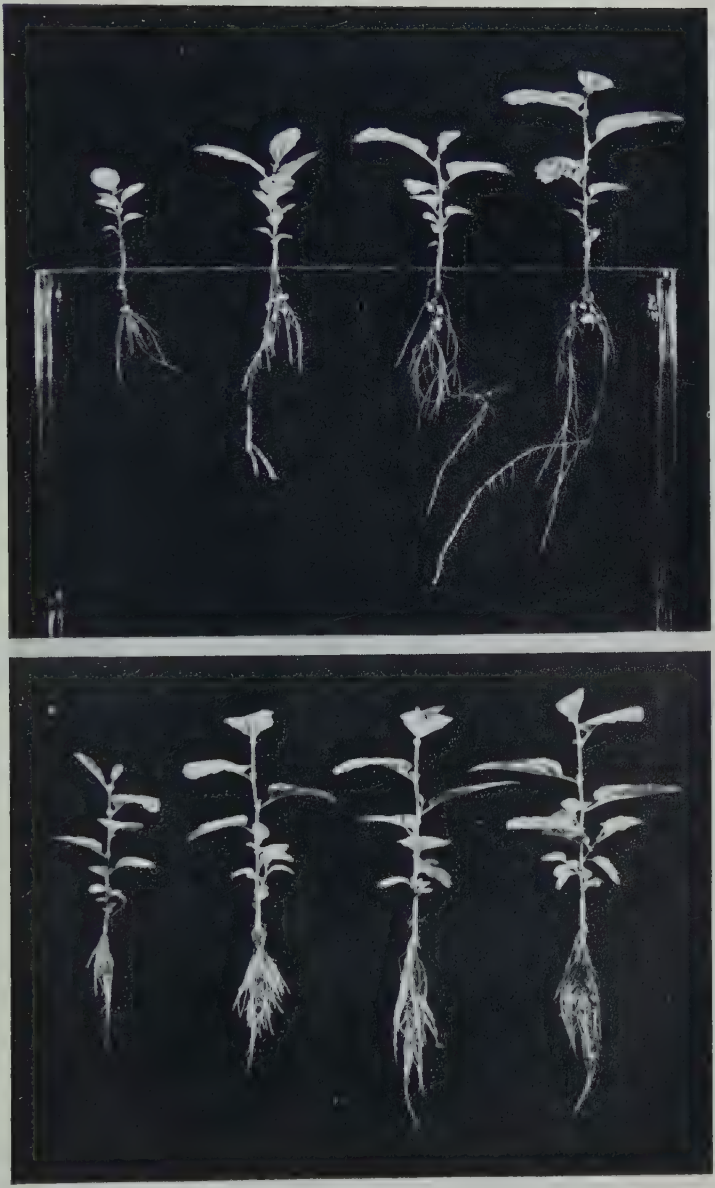
EXPLANATION OF PLATE

Illustrating the article by J. T. MacConnell on 'The Oxygen Factor in the Development and Function of the Root Nodules of Alder'.

FIG. 1. Typical nodulated alder plants the root systems of which were supplied with, from left to right, 1, 5, 10, and 21 per cent. oxygen respectively. (× 1/4)

FIG. 2. Typical non-nodulated alder plants grown in the presence of ample combined nitrogen and whose root systems were supplied with, from left to right, 1, 5, 12, and 21 per cent. oxygen respectively. (× 1/6)



J. T. MacCONNELL

Studies in the Growth of Young Plants of *Pinus sylvestris* L.

II. The Relation of Growth to Soil Moisture Tension

BY

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ABSTRACT

Plants of *Pinus sylvestris* in their first and third seasons of growth were grown in containers under moisture régimes in which the maximum tensions were varied between 0·1 and about 9 atm. Compared with plants growing in the 0·1 atm. treatment, growth was significantly reduced in the first-year plants by 0·3 atm. and in the third-year plants by a tension between 0·5 and 1·5 atm. Further reductions were caused by the higher tensions employed. The plants appeared to be sensitive to differences in soil moisture tension throughout the growing-season. The effects of soil moisture tension on growth were due mainly to variations in net assimilation rate. It is pointed out that the growth of many plants is substantially reduced by soil moisture tensions of less than 1 atm. and possible interpretations of this are discussed.

INTRODUCTION

IN the south-east of England where, compared with the rest of the British Isles, rainfall is relatively low (average summer rainfall varies from 25 to 30 cm.), *Pinus sylvestris* is notable as a species which readily forms natural woodlands, and which is also successfully planted on some of the lightest soils, especially on sandy heaths. While it is quite possible that this is to be explained primarily by a tolerance of poor nutritional conditions, it is clear that water relations may also be significant in a consideration of the autecology of this species. The experiments which are to be described represent an initial stage in the analysis of the relation of growth of *P. sylvestris* to soil moisture conditions.

There appears at present to be little information on the soil moisture requirements for the growth of this or any other conifer. Wenger (1952) found that the growth of first-year seedlings of *Pinus taeda* and *P. echinata* was inversely related to the percentage of water between the moisture equivalent and the permanent wilting percentage, and Negisi and Satoo (1954*a*, *b*) observed that apparent photosynthesis of seedlings of *P. densiflora* gradually declined as the soil dried below field capacity.

In the first paper of this series an investigation by growth analysis methods of the annual cycle of assimilation and growth in one- to five-year-old plants of *P. sylvestris* was described by Rutter (1957). A similar analysis has now been made using plants grown in varying conditions of soil moisture in pot culture.

[Annals of Botany, N.S. Vol. 23, No. 90, 1959.]

METHODS

Plants, pots, and soil. The plants used in Expts. 1 and 2 were transplanted at the end of their second year of growth into 8-inch diameter flower-pots. The pots were waterproofed by impregnation with wax and each was covered with a cone of stiff plastic which overlapped the rim of the pot and through the apex of which the stem of the plant passed. By this means rain was prevented from entering the pot and evaporation from the soil surface was reduced. In Expt. 3 plants were grown from seed, at a density of one per square inch in cans 7 inches in diameter, 10 inches deep, and perforated at the bottom. All the experiments were conducted in the open, and in Expt. 3 screens of transparent plastic were placed over the cans, in groups, during wet weather. Both pots and cans were plunged in soil.

The soil used was a sandy loam whose properties have been described elsewhere (Rutter and Sands, 1958). In the pots its maximum water content was about 30 per cent. of dry weight, and at 5 atm. tension (the maximum tension which could be measured by the method used) 4 per cent. remained in the soil.

Nutrients were added in solution to the pots and the quantities, relative to the maximum water content of the pot, were approximately:

Nitrogen, 200 p.p.m., as ammonium nitrate,
Phosphorus, 200 p.p.m., as dibasic sodium phosphate,
Potassium, 100 p.p.m., as potassium sulphate.

These concentrations are about two-thirds of those found by Mitchell (1939) to be optimal for the growth of *Pinus strobus* seedlings in water culture. Since, at watering, a small excess of water drained through the soil and out of the bottom of the pot, renewed applications of nutrients were given at intervals of 4 to 6 weeks. In Expts. 1 and 2, nitrogen was withheld from some pots, giving 'with' and 'without' nitrogen treatments.

Measurement of soil moisture tension. In all experiments, tensiometers with mercury manometers were used to measure moisture tensions up to 0·7 atm. Tensions from 1 to 5 atm. were measured in Expts. 1 and 2 with gypsum blocks which could be removed from the soil and weighed. The construction, use, and calibration of these blocks has already been described by Rutter and Sands (1958). In Expt. 3, electrical resistance blocks of Bouyoucos type, which also had been calibrated against tension, were used. In Expts. 1 and 2 only one instrument was placed in each pot, since previous experience had shown that the whole pot was quickly exploited by the roots of transplants and the distribution of moisture was fairly uniform. In Expt. 3, in which seedlings were grown in 10-inch-deep cans, tensiometers or resistance blocks were placed at 2, 4, and 8 inches depth and the average tension within the depth exploited by roots at any time was recorded.

Soil-water treatment. The pots were allowed to dry to a pre-determined tension before being re-saturated, and the treatments differed in the maximum tension allowed. The frequency of watering in different treatments is shown in Table 1. The wettest treatments had to be watered every few days, and almost every day at the hottest times of the summer.

TABLE 1

Frequency of Watering in Different Treatments, Relative to the Driest Treatment

	Max. moisture tension, atm.							
	0·1	0·3	0·5	1·0	1·5	3·0	> 5·0	9·0
Expt. 1	—	—	4	—	2	—	1	—
2	17	—	8	—	2	—	1	—
3	14	7·5	—	3·5	—	2	—	1

Design of experiments. In Expt. 1, 96 plants were divided into 8 groups, each consisting of 12 plants of similar size, as judged by measurements of height and stem diameter. Each group constituted an experimental replicate and in this way most of the variance due to initial differences in the size of the plants was removed from the treatment comparisons. Within each group three soil water treatments, with maximum tensions of 0·5, 1·5, and > 5·0 atm., were factorially combined with treatments with and without additional nitrogen. The criterion for the driest treatment was that the gypsum blocks reached their air-dry state. The corresponding tension could not be accurately characterized, but exceeded 5 atm. Watering control began on June 7; a sample of half the plants was taken on July 17 and a second sample on September 4.

Expt. 2, which was carried out on a much larger scale, included one wetter treatment than in Expt. 1 and was also designed to test the effect of varying the length of the period in which differential treatments were applied. Three 7-week periods of growth were distinguished, viz.

(A) April 14 to June 1, during which stem extension was completed and leaf growth begun,
(B) June 1 to July 24, during which rapid leaf extension occurred, and
(C) July 24 to September 4, during which leaf extension was completed.

To one series of plants (series ABC) differential water treatments were applied throughout these three periods, and samples of 48 plants were taken on April 14, June 1, July 24, and September 4. Another series of plants (BC) received differential treatment during the middle and last of the periods defined above, and a third series (B) during the middle period only. When these series were not receiving differential treatment they were kept at water tensions below 0·5 atm. A sample of 48 plants was taken on June 1 to determine the weight of plants in series B and BC before treatment began, and a similar sample on July 24 when they had received 7 weeks' treatment. Finally, two further samples of 48 plants were taken on September 4, one from series B and one from BC. Thus 8 samples of 48 plants, 384 plants in all, were taken throughout the course of the experiment. Within each sample, four water treatments, with maximum tensions of 0·1, 0·5, 1·5, and > 5·0 atm., were factorially combined with two nitrogen levels, in sixfold replication. Initial differences in plant size were again the basis of the division of the plants between the replicates.

Expt. 3 was carried out with plants grown from seed. It was not originally conceived in relation to Expts. 1 and 2, but in spite of the marked differences in organization between first-year seedlings and plants in their third year, all three experiments have given somewhat similar results and are conveniently described together. Working with first-year seedlings has the great advantage that large numbers can be grown together in one container and errors due to initial differences in plant size are far less than in the experiments in which only one plant can be grown in each pot. On April 7 thirty-five seeds per can were sown on the moist surface of the soil, carefully spaced, and covered with about 0·5 cm. Bedford sand. During the period of germination and emergence, until June 10, they were given either a Wet or Dry Emergence Treatment, i.e. they were lightly watered either every 4 or every 8 days. The surface soil of the cans appeared to dry more rapidly than would a well-prepared seedbed and emergence in the Dry Treatment averaged only 15 per can; in the Wet Treatment it was 25 per can. A sample was taken on June 10 and from this date to September 24, five Growth Treatments with maximum tensions 0·1, 0·3, 1·0, 3·0, and 9·0 atm. were factorially combined with the two Emergence Treatments. The experiment was performed in threefold replication; 30 cans were taken as samples on August 18 and 30 on September 24.

Sampling procedure. The soil was carefully washed from sampled plants and they were divided into root and shoot, and the shoot further subdivided into the stem and leaves of each year's extension growth. Small subsamples were taken from each leaf age for the determination of the area: dry weight ratio by the method described by Rutter (1957). All parts were dried to constant weight at about 80° C. in an oven with moving air-stream.

RESULTS

Stem extension. In Expt. 1 treatment did not begin until June 7, when extension of the apical shoot was almost completed, and in Expt. 2 little further apical extension took place after June 1, when series BC and B were first subjected to differential treatment. Table 2 shows that in series ABC, which were treated from April 14, there was a reduction in the extension of the apical shoot caused by the drier soils.

TABLE 2

Experiment 2. The Relation between Soil Moisture Conditions and the Final Length of the Apical Shoot in Plants Treated from April 14

Means of 24 plants

Max. tension, atm.	0·1	0·5	1·5	> 5·0
Apical shoot length, cm.	17·6	16·7	13·6	13·5

Sig. diff. ($P = 0{\cdot}05$) = 1·7

Leaf extension. The effect of soil moisture conditions on the mean length at the end of the season of needles on the apical shoot is shown in Table 3.

TABLE 3

The Relation between Soil Moisture Conditions and the Final Length of Needles on the Apical Shoot

Mean needle length, cm.

	Period of treatment	Max. soil moisture tension, atm.				Sig. diff. ($P = 0·05$)
		0·1	0·5	1·5	> 5·0	
Expt. 1	June 7–Sept. 4	—	5·85	4·80	3·75	0·65
Expt. 2	Apr. 14–Sept. 4	5·05	4·25	4·20	3·40	Interaction not significant
	June 1–Sept. 4	4·95	4·35	4·25	3·75	
	June 1–July 24	5·00	4·50	4·80	4·00	
Mean, all periods		5·00	4·35	4·40	3·70	0·45

Each successive increase in maximum soil moisture tension resulted in a decrease in the final needle length. Although the interaction of moisture level with period of treatment in Expt. 2 is not significant, there is a suggestion that the drier treatments had more marked effects the longer they were continued. The rather small differences between the different durations of treatment are probably due to the fact that all series received differential treatment in the period June 1 to September 4, when about half of the total leaf growth occurred. The leaves were measured at frequent intervals throughout the season and it was found that drought did not reduce the duration of leaf growth, but that in all treatments growth continued until the beginning of September, when the last measurements were made.

Annual dry-weight increment. The effects of treatment on the dry weights of the plants near the end of the growing season are shown, for Expts. 1 and 2, in Table 4. The analyses of variance were performed after transforming the dry weights to a logarithmic scale. The difference between the treatments with maximum tension of 0·1 and 0·5 atm. respectively (a comparison made only in Expt. 2) was not significant. Increasing the maximum tension from 0·5 to 1·5 atm. significantly reduced growth in both experiments, and an increase to 5·0 atm. produced a further reduction although this was not significant in Expt. 1.

TABLE 4

The Effect of Varying Soil Moisture Conditions on the Final Dry Weight in the Third Season of Growth

Wt. per plant, g.

	Period of treatment	Max. soil moisture tension, atm.				Sig. diff. ($P = 0·05$)
		0·1	0·5	1·5	> 5·0	
Expt. 1	June 7–Sept. 4	—	29·5	24·1	22·4	—
Expt. 2	Apr. 14–Sept. 4	30·2	26·7	19·6	18·2	—
	June 1–Sept. 4	33·2	30·8	28·4	22·6	—
	June 1–July 24	33·2	31·0	30·5	27·6	—
Mean, all periods		32·2	29·5	26·2	22·8	—
		Mean log. wt./per plant, g.				
Expt. 1	June 7–Sept. 4	—	1·442	1·370	1·333	0·072
Expt. 2	All periods	1·474	1·442	1·384	1·329	0·052

There was a significant interaction between intensity and duration of treatment in Expt. 2 which was most clearly shown if the treatments were grouped into two pairs, moist (0·1 and 0·5 atm.) and dry (1·5 and > 5·0 atm.) respectively (Table 5). It may then be seen that increasing the duration of the dry treatments increased their effect. With the shortest duration, July 1 to July 24, the differences between treatments were not significant although some reduction of growth by the drier treatments is suggested. Since the growth of the plants given dry treatments from June 1 to September 4 was significantly less than those treated only from June 1 to July 24, it is clear that they were affected by dry soil conditions in the period July 24 to September 4. Similarly the comparison of plants treated from April 14 to September 4 with those treated from June 1 to September 4 shows that they were sensitive to drought in the period April 14 to June 1. It therefore seems probable that drought may have an effect at any time in the growing season and that the effect on the plants treated from June 1 to July 24 is real, but fails to reach significance because of the relatively short duration of the treatments.

TABLE 5

The Interaction of Varying Soil Moisture Conditions with Duration of Treatment on the Final Dry Weight in the Third Season of Growth
(Expt. 2)

Means of final dry weight and log. final dry weight per plant, g.

Period of treatment		Max. soil moisture tension, atm.	
		0·1 and 0·5	1·5 and > 5·0
Apr. 14–Sept. 4	Dry wt.	28·5	18·9
	Log. dry wt.	1·431	1·262
June 1–Sept. 4	Dry wt.	32·0	25·5
	Log. dry wt.	1·464	1·372
June 1–July 24	Dry wt.	32·1	29·1
	Log. dry wt.	1·479	1·435

Sig. diff. ($P = 0·05$) between logarithms = 0·064

It must be remembered that the final weights shown in Tables 4 and 5 include a part of the plant which was formed during the 2 years preceding treatment. The initial weight in the year of treatment was not determined in Expt. 1 but in Expt. 2 it was 7·0 g. Thus in the series continuously treated, the dry-weight increment in the wettest treatment was about twice that in the driest.

An analysis was made of the course of dry weight increase throughout the season and the results of this are set in the form of relative growth-rates in Table 6.

In Expt. 1, the sampling times allowed the calculation of relative growth-rate during the latter part of the season only, viz. from July 17 to September 4, and during this period there was no effect of treatment, which suggested that the differences in final plant weight found in this experiment were due to differences in relative growth between June 7, at the inception of treatments, and July 17. In Expt. 2, in the series with continuous treatment, increase in

TABLE 6

The Effect of Varying Soil Moisture Conditions on Relative Growth Rate at Different Periods in the Third Season of Growth

Dry-weight increment, percent. per day

Period of treatment	Period of growth	Max. soil moisture tension, atm.				Sig. diff. ($P = 0·05$)
		0·1	0·5	1·5	> 5·0	
Experiment 1						
June 7–Sept. 4	July 17–Sept. 4	—	0·73	0·97	0·77	not sig.
Experiment 2						
Apr. 14–Sept. 4	Apr. 14–June 1	0·54	0·45	0·36	0·17	not sig.
	June 1–July 24	1·86	1·65	1·45	1·11	0·66
	July 24–Sept. 4	0·71	0·61	0·47	0·74	not sig.
June 1–Sept. 4	June 1–July 24	1·17	1·51	1·32	1·15	not sig.
	July 24–Sept. 4	1·15	1·08	0·86	0·50	not sig.

maximum soil moisture tension reduced the relative growth-rate in the first part of the season, the differences being significant between June 1 and July 24; but from July 24 to September 4 there were, again, no effects of treatment. In both these cases, then, there appeared to be some adaptation to treatment in that effects of treatment on relative growth-rate disappeared after the middle of July. However, in the series of Expt. 2 in which differential treatment was begun on June 1, and which was therefore comparable with Expt. 1, there appeared to be effects on relative growth-rate until the end of the season, although these were not significant.

Clearly, too much confidence should not be placed on the conclusions suggested by Table 5 since, in relation to the magnitude of the relative growth-rates, their errors are very high. Growth was slow and initial variation in the sizes of the plants contributed very much to the errors involved.

In Expt. 3, when the Wet and Dry Emergence Treatments were ended on June 10, the mean dry weights per seedling were 0·035 and 0·029 g. respectively. However, no difference between these treatments was found when the final dry weights were obtained on September 24. This is possibly to be explained by the greater numbers emerging and more intense competition resulting from the Wet Emergence Treatment. The relative growth-rates under the five Growth Treatments, which were applied from June 10, and the final weights attained by September 24, are shown in Table 7.

In this experiment each increase in maximum soil moisture tension caused a significant reduction in the final dry weight of the plant. The effects of treatment on relative growth-rate persisted throughout the season.

Net assimilation rate. In Expts. 1 and 2, with plants in their third year of growth, the variability of the plants and their slow rate of growth caused large errors in the calculated net assimilation rates. In Expt. 1 no significant effects were found between June 17 and September 4, the only period available for estimating assimilation rates. In Expt. 2, after pooling the data from the

TABLE 7

The Effects of Variation in Soil Moisture Conditions on Dry-weight Increment in the First Season of Growth

	Max. soil moisture tension, atm.					Sig. diff.
	0·1	0·3	1·0	3·0	9·0	(*P* = 0·05)
Relative growth-rate, percent. per day						
June 10–Aug. 18 .	4·16	4·07	3·54	3·22	2·97	0·32
Aug. 18–Sept. 24 .	1·64	1·52	1·32	1·12	1·00	0·59
Dry wt. per plant, g., Sept. 24						
	0·94	0·80	0·61	0·44	0·37	—
Mean log. dry wt. 10 plants, Sept. 24						
	0·97	0·90	0·78	0·64	0·56	0·06

continuously treated series and the series treated from June 1 to September 4, and calculating the net assimilation rate over this period, a significant reduction with increasing soil moisture tension was found (Table 8).

TABLE 8

The Effects of Varying Soil Moisture Conditions on the Net Assimilation Rate of Plants in the Third Season of Growth

g. per dm.^{2} per week

		Max. soil moisture tension, atm.				Sig. diff.
Expt.	Period	0·1	0·5	1·5	>5·0	(*P* = 0·05)
1	June 17–Sept. 4	—	0·203	0·260	0·198	not sig.
2	June 1–Sept. 4	0·414	0·393	0·342	0·308	0·077

In Expt. 3, carried out with first-year seedlings, far more plants contributed to each estimate of net assimilation rate and the effects of soil moisture treatment were of similar magnitude to those shown by Expt. 2, but were highly significant (Table 9).

Some explanation is needed of the significant interaction between Emer-

TABLE 9

The Effects of Varying Soil Moisture Conditions on the Net Assimilation Rate of Plants in the First Season of Growth

g. per dm.^{2} per week

	Growth treatment Max. soil moisture tension, atm.					Sig. diff.
Emergence treatment	0·1	0·3	1·0	3·0	9·0	(*P* = 0·05)
June 10–Aug. 18						
Wet . . .	0·322	0·312	0·280	0·258	0·236	Interaction not sig.
Dry . . .	0·371	0·353	0·302	0·286	0·277	
Mean . . .	0·347	0·333	0·291	0·272	0·257	0·040
Aug. 18–Sept. 4						
Wet . . .	0·153	0·171	0·199	0·127	0·039	0·072
Dry . . .	0·249	0·206	0·156	0·080	0·111	
Mean . . .	0·201	0·189	0·178	0·104	0·075	0·051

gence Treatment and Growth Treatment in the values for August 18 to September 24, when the effect of increasing soil moisture tension in the Dry Emergence series was a steady decrease in net assimilation rate, whereas in the Wet series there was no significant effect until the tension exceeded 1 atm. The explanation of this probably lies in the much higher density of seedlings resulting from the Wet Emergence Treatment and the greater competition for light which ensued. The intensity of this competition may be gauged from the leaf-area index (Watson 1947), i.e. the ratio of leaf area to soil area. Mean values of leaf-area index for the periods used in the calculation of net assimilation rate are shown in Table 10. It will be seen that in the period June 10 to August 18, at any level of soil moisture the higher values of net assimilation rate resulting from the Dry Emergence Treatment are associated with lower values of leaf-area index. Leaf-area indexes were generally less than 2. In the period August 18 to September 24 leaf-area indexes in the Wet Emergence series increased from 2·6 in the 1·0 atm. treatment to 4·0 in the 0·1 atm. treatment and it is suggested that the great density of foliage in the last treatment prevented the increase in net assimilation rate which might have been expected to result from the free moisture supply. In the Dry series and at the same soil moisture levels, leaf-area indexes were 2·5 or less and net assimilation rate increased significantly with soil moisture.

TABLE 10

The Effects of Varying Soil Moisture Conditions on Leaf-Area Index in Expt. 3

Leaf area per unit soil area

	Growth treatment Max. soil moisture tension, atm.				
Emergence treatment	0·1	0·3	1·0	3·0	9·0
June 10–Aug. 18					
Wet	2·2	2·1	1·5	1·2	1·0
Dry	1·2	1·0	0·9	0·7	0·6
Aug. 18–Sept. 24					
Wet	4·0	3·8	2·6	1·9	1·5
Dry	2·5	2·0	1·5	1·1	0·9

An inverse relation between net assimilation rate and leaf-area index, such as has been invoked to explain the results of Expt. 3, has previously been demonstrated by Watson (1956) in kale and sugar-beet.

Relative leaf growth. In the plants in their third year of growth, variation in soil moisture conditions had very little effect on the expansion of leaf area relative to the growth in weight of the plant. A selection of the results of Expt. 2 is given in Table 11. The partition of the dry-weight increase between the new foliage and the rest of the plant was first examined and was found to be unaffected by soil water treatment (Table 11, *a*). Since, however, the total dry-weight increment was reduced in the drier soils so also the weight of new leaves produced relative to the weight of the whole plant was also reduced.

However, the ratio of area to dry weight in the new leaves significantly increased with soil moisture tension (Table 11, *b*) with the result that the ratio of the area of new leaves to the weight of the whole plant (new-leaf-area ratio) was almost the same at all levels of soil moisture (Table 11, *c*). The data in the table represent the average of the two series with durations of treatment April 14 to September 4 and June 1 to September 4. If the results of the first of these series is separated a slight, but not significant, reduction in new-leaf-area ratio is found in the driest treatment.

The area per unit weight of the older leaves, which were formed in the year before the experiment was carried out, was unaffected by moisture treatment, but there was a tendency for these leaves to fall earlier in the driest treatment. This, combined with the slight depression of new-leaf-area ratio in the driest treatment, already referred to, produced a significant depression in total-leaf-area ratio in the series of plants with longest duration of treatment, viz. April 14 to September 4 (Table 11, *d*). This effect was small and only just significant at the probability level 0·05 and its importance is doubtful especially since it was not found when the duration of treatments was June 1 to September 4, nor in Expt. 1 with duration of treatments from June 7 to September 4.

TABLE 11

The Effect of Variation in Soil Moisture Conditions on the Development of Foliage in the Third Season of Growth

	Max. soil moisture tension, atm.				Sig. diff.
	0·1	0·5	1·5	> 5·0	($P = 0·05$)
(*a*) *Wt. new foliage: wt. increment of plant*					
June 1	0·46	0·43	0·56	0·42	not sig.
July 24	0·58	0·57	0·56	0·55	
Sept. 4	0·53	0·47	0·52	0·48	
(*b*) *Area: wt. of new leaves, dm.² per g.*					
July 24	1·60	1·65	1·71	1·86	—
Sept. 4	1·39	1·56	1·52	1·74	—
Mean	1·49	1·61	1·61	1·80	0·09
(*c*) *New-leaf-area ratio, dm.² per g.*					
July 24	0·62	0·64	0·64	0·59	not sig.
Sept. 4	0·62	0·59	0·57	0·64	
(*d*) *Total-leaf-area ratio, dm.² per g. in plants treated from Apr. 14 to Sept. 4*					
June 1	0·65	0·61	0·59	0·56	—
July 24	0·76	0·79	0·75	0·73	—
Sept. 4	0·69	0·69	0·71	0·64	—
Mean	0·70	0·70	0·68	0·64	0·04

In Expt. 3, where the plants were grown from seed, very little dry weight or foliage was produced before the beginning of treatments on June 10. In the ratio of leaf weight to plant weight (Table 12, *a*) there was an interaction with time in that the leaf-weight ratio was depressed by increased soil moisture tension in the sample of August 18, but not in the sample of September 24. In contrast to the older plants of Expts. 1 and 2, the area per unit weight of these first-year seedlings was depressed by the higher soil moisture tensions (Table 12, *b*), and in consequence the leaf-area ratio was also significantly lowered (Table 12, *c*).

TABLE 12

The Effects of Variation in Soil Moisture Conditions on the Development of Foliage in the First Season of Growth

	Max. soil moisture tension, atm.					Sig. diff. ($P = 0·05$)
	0·1	0·3	1·0	3·0	9·0	
(*a*) *Leaf wt. ratio, g. per g.*						
Aug. 18	0·635	0·622	0·625	0·612	0·585	0·035
Sept. 24	0·500	0·500	0·470	0·490	0·523	
(*b*) *Area: wt. of leaves, dm.^{2} per g.*						
June 10	← 1·98 →					—
Aug. 18	1·12	1·14	1·06	1·04	1·03	—
Sept. 24	0·95	0·95	0·90	0·94	0·83	—
Mean, Aug. and Sept.	1·03	1·04	0·98	0·99	0·93	0·06
(*c*) *Leaf-area ratio, dm.^{2} per g.*						
June 10	← 1·33 →					—
Aug. 18	0·70	0·71	0·66	0·64	0·60	—
Sept. 24	0·48	0·48	0·42	0·45	0·43	—
Mean, Aug. and Sept.	0·59	0·59	0·54	0·54	0·52	0·04

The conclusion drawn from the foregoing analysis of growth was that the ratio of leaf area to plant weight was very little affected by variation in water-supply, except perhaps in the first-year seedlings, and that the differences in growth were mainly due to effects on net assimilation rate. The importance of water-supply in net assimilation rate has already been demonstrated, in sugar-beet, by Morton and Watson (1948).

Effects of nitrogen. In Expts. 1 and 2, treatments with and without additional nitrogen were factorially combined with the soil moisture treatments. The soil used appeared, however, to contain a satisfactory, though not optimum, content of nitrogen in relation to the needs of the plants. Additional nitrogen increased the final dry weight by about 20 per cent. in Expt. 1 and 30 per cent. in Expt. 2. There was no significant interaction with soil moisture level and the effects of nitrogen have therefore not been shown in the tables. No effect of nitrogen on net assimilation rate was found but there was an effect on leaf-area ratio, sufficient to account for the observed differences in final dry weight.

DISCUSSION

Discussion of the effects of variation in soil moisture on the growth of plants was for many years dominated by the view of Veihmeyer and Hendrickson (1950) that between field capacity and the permanent wilting percentage (the so-called available range) variation in soil moisture had no effect on growth. Much evidence to the contrary has now accumulated and has recently been summarized by Stanhill (1957). It should be noted, however, that Majmudar and Hudson (1957) have shown for winter lettuce, and Salter (1957) for tomato that while the roots of these plants are rapidly extending into moist soil, growth is not affected by the depletion of water in the surface horizons, and this may explain the absence of effects on growth in some of the earlier experiments with other plants.

If it is accepted that growth may vary with soil moisture within the available range, then the expression of soil moisture as a percentage of the weight of the soil or of the total available moisture is unsatisfactory for many purposes since it gives no information on the state in which the water exists or the ease with which the plant may obtain it. It seems more satisfactory to characterize moisture by its tension although this also is an incomplete expression of the properties of the soil moisture which may affect growth. For while it shows the reduced pressure against which the plant must work, it neglects osmotic effects and gives no indication of the amount of water which may be obtained in a given tension range nor of the resistance to water movement from soil to plant.

Experiments on the relation of growth to soil moisture conditions are complicated by the impossibility of maintaining constant conditions or of distributing limited additions of water throughout the volume of soil occupied by the roots. In consequence nearly all previous investigators have adopted the course which was followed in the experiments described in this paper of allowing soil moisture to be depleted to predetermined levels and then restoring to field capacity before allowing drying to proceed again.

Care must then be exercised in comparing the results of experiments on the relation of the growth of different plants to soil moisture conditions, firstly because there is no entirely satisfactory way of characterizing the conditions at any one time, secondly because a statement of the maximum tension or minimum moisture content in a soil moisture régime is an incomplete description of the whole régime and thirdly because the effect of given soil moisture conditions will almost certainly be modified by the atmospheric conditions, especially those regulating transpiration rate. With these reservations, the present series of experiments may be compared with similar ones carried out in the past, i.e. with experiments whose moisture régimes have been characterized by the maximum moisture tension attained. Experiments have already been cited in which plants were insensitive to soil moisture depletion apparently because the roots were continuously extending into soil at field capacity, but in almost all experiments in which growth has been restricted by soil moisture depletion—and these, which form the majority of cases, include experiments in field conditions as well as in containers—it is observed that dry-weight increase or crop yield are appreciably restricted by tensions of about 0·5 atm. Examples of tensions which have been found to reduce growth significantly are, for potatoes 0·5 atm. (Howe and Rhoades, 1948), for maize 0·7 atm. (Haynes, 1948), for young apple-trees 0·55 atm. (Kenworthy, 1949), for sugar-cane 0·65 atm. (Waterhouse and Clements, 1954), for alfalfa 0·6 atm. (Stanberry *et al.*, 1955), and for tomatoes, after an initial insensitive phase of growth, 0·4 atm. (Salter, 1957). The comparisons which were made differed from experiment to experiment but compared with treatments freely watered or maintained near field capacity the above treatments mostly caused reductions of growth or yield in the range 10–20 per cent.

Turning to the experiments with *P. sylvestris* it was found in Expt. 2 that the treatment with maximum tension 0·5 atm. when compared with 0·1 atm. caused an apparent reduction in dry-weight increment of 15 per cent. if the treatments were continued throughout the season. This reduction was not significant, but it appeared consistently in the three series with different durations of treatment. Increasing the maximum tension to 1·5 atm. resulted in a further significant fall of 30 per cent., i.e. to 55 per cent. of the weight at 0·1 atm. so that it is clear that dry-weight increment began to fall sharply at tensions not much exceeding 0·5 atm. In Expt. 3 the final dry weight of first-year seedlings was significantly reduced by 15 per cent. between treatments with maximum tensions of 0·1 and 0·3 atm., and by a further 20 per cent. between 0·3 and 1·0 atm.

The various experiments cited were not carried out under sufficiently standardized conditions to permit a comparison of the sensitivity of the various species involved to water stress, but it seems remarkable that in all the species considerable restrictions of growth were caused by moisture tensions of about 0·5 atm., tensions which are very low in comparison with the suctions which plants are able to exert and which have frequently been found to be of the order of 10 atm. or more. We have found that needles of *P. sylvestris* can develop suctions of at least this value. Two comments seem appropriate. The first is that if such low tensions can restrict growth it is unjustifiable to neglect the osmotic pressure of the soil water, which may be of comparable magnitude. The second is that it may be mistaken to assume that measurements of soil moisture tension made by conventional means provide a satisfactory indication of the capillary forces opposing the plant's suction for water. It is well known that the permeability of the soil to water is inversely related to its water content, and data brought together by Richards and Wadleigh (1952) show that in most soils permeability suddenly declines to almost immeasurable values at tensions of about 0·2 atm. The marked restriction of growth which has been found in many plants to occur just above this level of tension may be due to the fact that the resistance to the withdrawal of water from the soil rises steeply, with the consequence that the plants must develop high suctions in soils whose opposing tensions do not appear to be great. It is suggested that, under the conditions of very low permeability to which reference has been made, the soil moisture tension at the absorbing root surfaces will be considerably higher than that in the general body of the soil, as shown by tensiometers and resistance blocks. One way of testing this hypothesis would be by experiments in which osmotic pressure and soil water tension were varied independently. Very little of the work on the effect of the osmotic pressure of culture solutions seems to have been concerned with the range 0 to 1 atm., although Gingrich and Russell (1957) have found that within this range osmotic pressures have less markedly depressing effects on the growth of maize seedlings than the equivalent soil water tensions. Since their seedlings were grown for only 24 hours and in completely closed containers which must have almost prevented transpiration and water demand, their experiments can only be considered an initial step in the comparison of osmotic and moisture tension effects.

As a contribution to the autecology of *P. sylvestris* the value of the present experiments is limited. It has been shown that this species, like many others, is sensitive to quite low moisture tensions and that this sensitivity is shown throughout the season of active growth. But the pot cultures which have been described differ from natural conditions in many respects, including several which may be all-important to the plant's water relations. In the first place, although the plants made quite normal shoot growth their roots were undoubtedly crowded. The volume of soil in each pot was approximately 3 litres and the dry weight of foliage of the 3-year-old plants at the end of the season was 7·5 g. in the driest and 15 g. in the wettest treatment; hence each gram of foliage could draw water from between 200 and 400 ml. of soil. Young plants of *P. sylvestris* in nature are characterized by rapidly extending horizontal roots, and even in woodland conditions of dense stand and closed canopy a greater volume of soil per unit weight of foliage has been observed. In a particular plantation with 10,000 Kg. dry foliage per hectare the roots densely filled the upper 60 cm. of the soil and were uniformly if more sparsely distributed through the next 60 cm. The volume of soil per unit weight of foliage was thus 1·2 litres. Moreover in closed canopy the exposure of the foliage to insolation and wind is less than in isolated plants in pots, and the demand for water for transpiration is probably lower. Thus several factors combined to cause a demand for a more rapid movement of water from the soil to the roots in the pot experiments.

In passing it may, however, be noted that the rooting conditions of the 1-year-old seedlings in 25 cm. deep cans were more closely comparable to those in forest nursery seed-beds.

There is a further criticism of these experiments as a means of investigating the ecological effects of soil moisture which applies also to most previous work. It is that the water régime in which the soil is allowed to dry while protected from rain, and is then brought to field capacity throughout the rooting range, is not at all readily compared with soil conditions in nature where the roots of a plant may be distributed between horizons with markedly contrasted soil moisture conditions, in respect both of the maximum tensions attained and the degree of repletion which occurs as a result of summer rains. The simple moisture régime which has been used in the pot experiments would appear to be a necessary first step in the analysis of the ecological importance of soil moisture, but attention must be turned to the significance of more complex patterns of soil–water distribution.

As a result of the foregoing experiments it has been concluded that growth analysis techniques are in many ways unsuitable for the analysis of the relation between soil moisture and growth, especially since the period between samples can hardly be shorter than one drying-cycle, and in a slow growing species like *P. sylvestris* it may perforce cover several cycles of wetting and drying. It seems desirable to follow the changing response of the plant within a cycle of drying and rewetting and since it has now been shown that the main growth responses of *P. sylvestris* to changes in soil moisture are caused through variation in the assimilation rate, experiments have been initiated in which photosynthesis is directly measured by a gas-exchange method.

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On *Eosperma oxroadense* gen. et sp. nov.: a New Lower Carboniferous Seed from East Lothian

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With one Plate and three Text Figures

ABSTRACT

A new petrified platyspermic seed is described from the Cementstone Group (Tournaisian) of Scotland. It has a generally Lagenostomalean appearance despite its unusual symmetry, and it is compared with some other members of that order. It possesses a well-preserved megaspore membrane, one of the most notable features of which is the polygonal markings on the outer surface. Comparison is made with some megaspores from fossil and living seeds. The possible affinity of the seed is discussed.

MATERIAL AND METHODS

THE material used was collected by the late Professor W. T. Gordon, and is now in the collection of the Geological Department, King's College, London. It is a nodular infilling of volcanic ash containing petrified plant remains, embedded in the surface of a minor disconformity of the main beds of ash comprising the cliffs of Oxroad Bay just south of Tantallion Castle, East Lothian, Scotland.

Gordon described from this source a stem, *Tetrastichia bupatides* (1938) and later a seed, *Salpingostoma dasu* (1941). These were not all the plant remains contained in the ash (as Gordon himself pointed out) and other fossil plants still remain to be described. Seeds occur throughout the material and their frequency in block 125 from which most of the specimens have been obtained is about one per c.c. The total number of seeds seen exceeds 200; of these over three dozen have been serially sectioned and a further two dozen macerated.

The structure of these seeds has been investigated from petrological sections in the Gordon collection, from serial peel sections, and from cuticle preparations. Despite Gordon's comments (1941) that the peel technique of Walton (1928) was unsatisfactory, I have found it work quite well. The method used has been that of Lacey *et al.* (1956) with 2–3 per cent. hydrochloric acid as the etching medium. The cuticle preparations were obtained by breaking down the rock in concentrated hydrochloric acid and removing the cuticles. These were then freed of adhering siliceous fragments by treatment in hydrofluoric acid and finally macerated in nitric acid followed by dilute ammonia. They were mounted either in glycerine jelly or, after dehydration, in Canada balsam.

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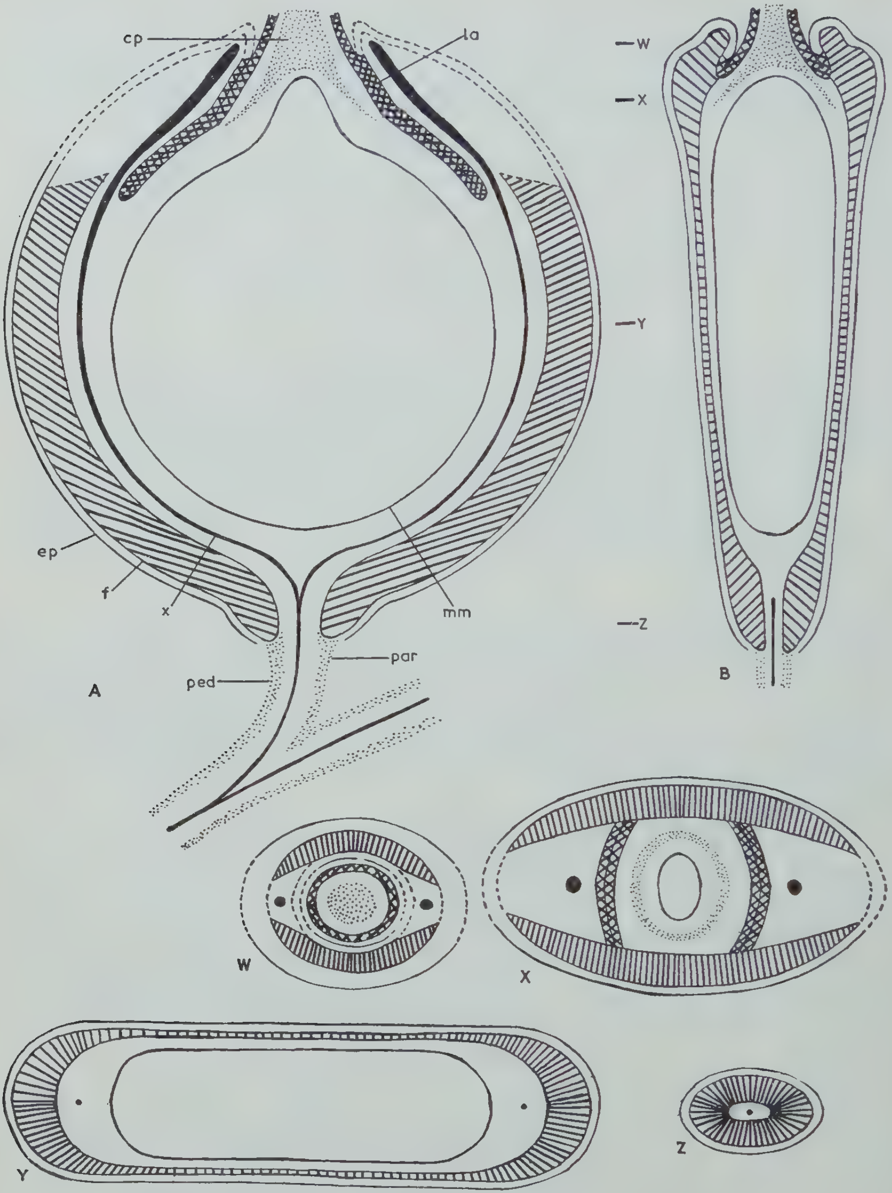
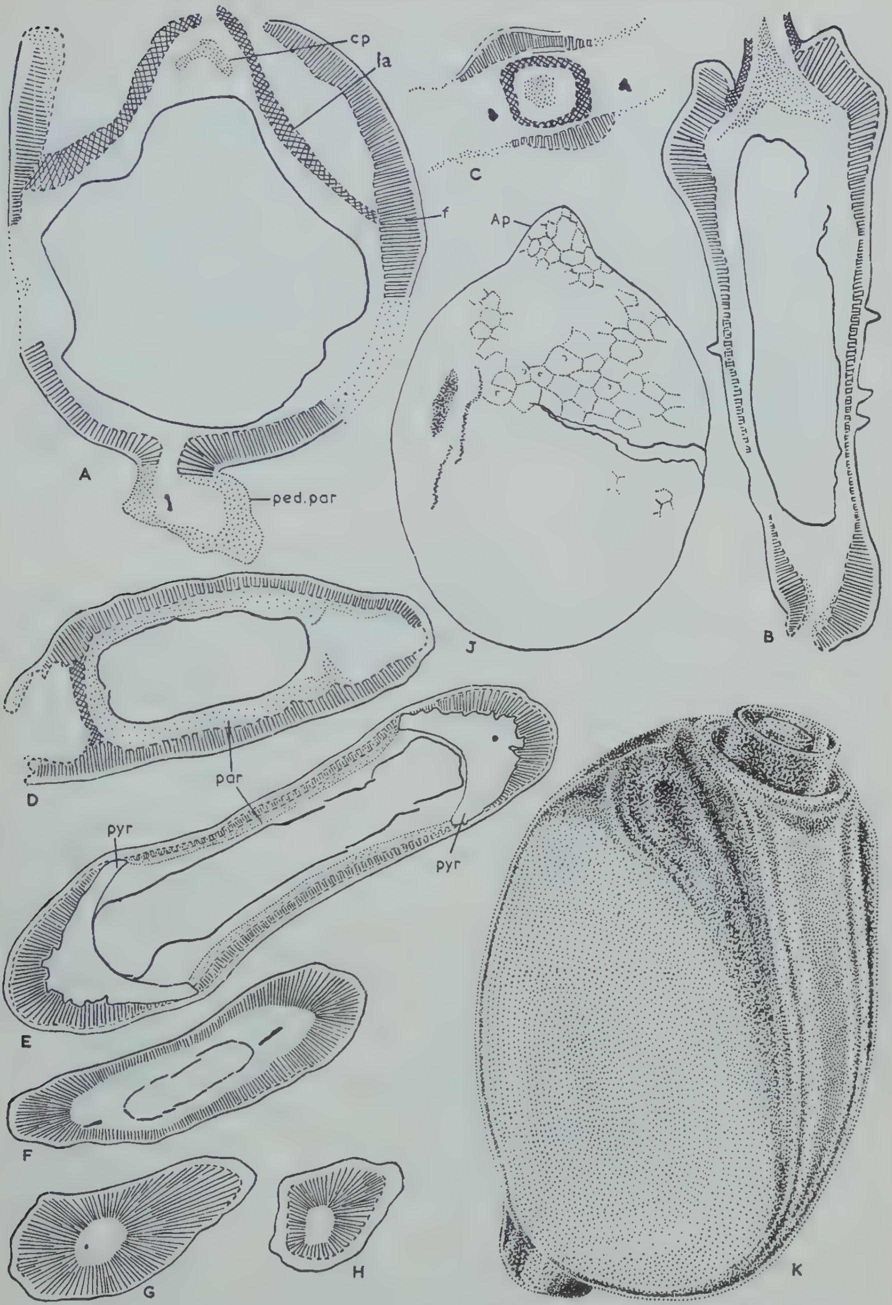


TEXT-FIG. 1. *Eosperma oxroadense* gen. et sp. nov. Reconstructed sections. A, longitudinal section in major plane, B, in minor plane; W–Z, transverse sections in the planes indicated. *cp* = central column of parenchyma, *ep* = epidermis, *f* = fibres, *la* = lagenostome, *mm* = megaspore membrane, *par* = parenchyma, *ped*= pedical, *x* = xylem. All ×25 (approx.).



Text-fig. 2. *Eosperma oxroadense* gen. et sp. nov. Camera lucida drawings of sections comparable to those shown in text-fig. 1; A, in major plane (seed β 10) G.C. 2135 (14), and B, in minor plane (seed β 2) G.C. 2154; transverse sections, C, apical (seed α 19) G.C. 2147 (49c), D, sub-apical (seed α 4) G.C. 2113; E, median rock section, *pyr* = pyrite, G.C. 2036; basal series (seed α 3) F, G.C. 2114 (10), G and H, G.C. 2115 (14 and 16). J, megaspore, *Ap* = apical papilla (G.C. 2188 before dissection). K, drawing of a plaster model of *Eosperma oxroadense*. Shading and symbols as in text-fig. 1. All ×25.

GENERAL FORM

A typical seed is bilaterally symmetrical and greatly flattened in one plane. The outline in the major plane is nearly circular (Text-fig. 1 A) but that in the minor plane is nearly cuneiform (Text-fig. 1 B) with the apical end about twice as thick as the base. There is frequently some degree of curvature in the minor plane. At the base there is a small papilla by means of which the seed was attached to a pedicel; only seven specimens have been found attached. At the apical end there is exposed the protruding tip of what is assumed to be the pollen-catching apparatus. This consists of two parts: a central column and, surrounding it, the lagenostome (Plate, Fig. 1).

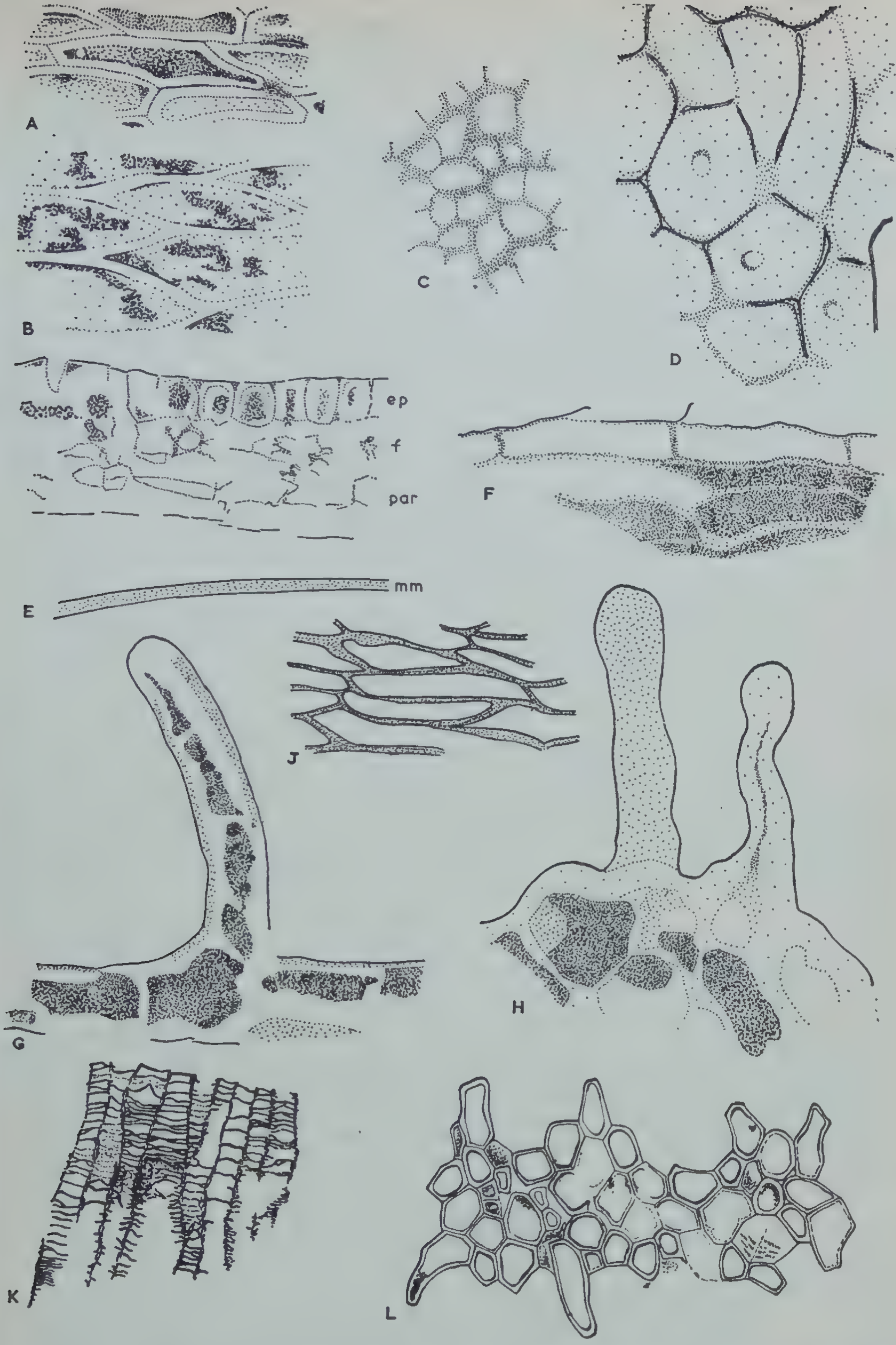
The seeds occur in two distinct forms, *forma* α with a smooth surface and *forma* β which is covered with unicellular epidermal hairs. This character makes it possible to distinguish between the two forms as seen on the surface of a cut block by using only a hand lens. Of these two forms, α occurs with a frequency ten times that of β.

STRUCTURE

a. The integument. Surrounding the megaspore is an integument. It is bounded by an epidermis of longitudinally elongated cells which when well preserved show thickening on the radial and external walls (Text-fig. 3 E). The hairs present in forma β (Text-fig. 3 G, H) are often black and opaque but some are transparent amber. The presence of a thin cuticle is suggested in some of the rock sections but it does not show up distinctly in the peels, nor has it been possible to demonstrate it by maceration. The approximate measurements of the epidermal cells are:—length, α 150 μ, β not determined; radial walls, α 20–40 μ, β 40–60 μ; tangential walls, α 30–40 μ, β 60 μ; hairs on β 100–300 μ long by 40 μ in diameter.

Within the epidermis there is a layer of fibrous tissue, with cells elongated longitudinally never radially (Text-fig. 3 J, F). In the majority of the seeds this tissue is all that is preserved and even this may be represented only by granules of a black coaly substance. Towards the apex the fibrous tissue forms a conspicuous thickening on either side in the minor plane. It is this which is largely responsible for the increase in thickness of the seed in the sub-apical region. Here the tissue is ten to fifteen cells deep but lower down the flattened sides of the seed it thins out probably to a single layer; but the preservation is such that the individual cells in this region are seldom apparent. In the lower two-thirds of the seed the fibrous tissue is thick in the two edges as seen in transverse section. The approximate dimensions of these cells are: length, 150–216 μ; radial width, 10–30 μ; tangential width, 15–40 μ.

The centre of the seed is filled with a lens-shaped mass of calcite. This usually appears to have replaced the contents of the megaspore or female gametophyte, the membrane of which usually surrounds the calcite. In many seeds, however, the membrane is in a collapsed and contracted state inside the calcite which would thus then appear to fill the space previously occupied by



TEXT-FIG. 3. *Eosperma oxroadense* gen. et sp. nov. Details of tissue; A, lagenostome in tangential longitudinal section (seed α 20) G.C. 2157 (13); B, epidermal cells in surface section (seed β 6) G.C. 2131 (39); C, gametophyte tissue in tangential longitudinal section (seed β 4) G.C. 2177 (14); D, surface view of megaspore membrane showing polygonal reticulations with attached cuticle fragments and semilunar markings, G.C. 2188; E–H, epidermis from rock sections; E, in transverse section, forma α, G.C. 2036; H, forma β, G.C. 1994; F, radial longitudinal section, forma α, G.C. 2041; G, forma β, G.C. 1995; J, fibrous cells in radial longitudinal section (seed β 6) G.C. 2130 (29); K, tangential longitudinal section of the xylem trace in the sub-apical region (seed β 3) G.C. 2172 (5); L, transverse section of the xylem trace just below the apex with some of the outermost tracheids elongated radially (seed β 5) G.C. 2171 (4). Symbols as in Text-fig. 1. All ×150 except D ×100 and K and L ×400.

the megaspore. The region between the megaspore cavity and the subepidermal fibres is filled either by an acellular shell of mineral (pyrite or ashy matrix) or this may contain some remains of a cellular tissue, apparently parenchyma (Text-fig. 3 E). The cells of this tissue lying immediately underneath the fibres are more elongated (160 μ) and narrower (20 μ) than the deeper lying cells (120 by 50 μ).

b. The vascular system. The vascular supply within the pedicel consists of a single strand which dichotomises after it has entered the basal papilla to produce two traces which pass up within the parenchyma just underneath the fibres, one in each edge of the seed (Text-fig. 1 A). Usually the strand in the papilla is represented by only a few (1 to 6) tracheids, although in seed no. α 21 there are twelve or more tracheids in this region. As the traces run towards the apex, they increase in size so that in the apical transverse sections there are twelve to thirty-six tracheids per trace (Text-fig. 3 L and Plate, Fig. 6). At the apex of the seed some of the tracheids as seen in longitudinal sections appear to have rounded ends which are at approximately the same level. The tracheids are mainly scalariform (Text-fig. 3 K) though there are sometimes some of the spiral or annular type. The average tracheid diameter is 13 μ, the minimum value 8 μ, and the maximum 20 μ. The position of the protoxylem has not been demonstrated with certainty, but it is probably mesarch. It has not been possible to distinguish any phloem.

c. The megaspore. The megaspore has the form of a moderately flattened pear-shaped body (Text-fig. 2 J). The membrane as seen in surface view is spongy in texture, appearing to be composed of a three-dimensional reticulum with interstices of less than 1 μ (Plate, Fig. 5). In specimens where the megaspore membrane has become torn during maceration it may sometimes be seen to have separated at the torn edge into two layers. Microtome sections of macerated megaspores show the membrane to be composed of three layers: inner and outer spongy layers of about equal thickness, and a slightly thinner, dark but more or less homogeneous middle layer (Plate, Fig. 4). In intimate contact with the outer surface of this whole membrane, which I take to be the megaspore membrane itself, there is a further cutinized reticulum of large dimensions which gives a cellular appearance to the macerated megaspore coat (Text-fig. 3 D). The polygonal areas of this network are about 50–100 μ by 100–250 μ. The polygons vary in shape from nearly isodiametric to markedly elongate; where elongate their long axes would appear to be orientated parallel to the direction of growth of the surface of the flattened spore. The reticulum appears to consist of a shallow open honeycomb, and there is no indication of a continuous membrane across the network parallel to the surface of the megaspore proper. As seen at the edges of the megaspore the thin lamellae forming the reticulum stand out to about 30 μ from the megaspore membrane. This cuticle is a clear pale yellow and exhibits no fine structure; it is usually less than 3 μ thick. In one of the spores there are crescentic marks in the centre of some of the polygonal areas, which may possibly represent compressed papillae belonging to the megaspore membrane.

They are slightly more translucent than the rest of the membrane and may be somewhat thinner or structurally different.

The calcite-filled cavity within the membrane is devoid of organic contents except in seed β 4. In this specimen there is present the remains of some regular polygonal parenchyma (20–40 μ) (Text-fig. 3 C) which possibly represents a portion of the original female gametophyte.

d. The basal pole. The basal papilla is formed largely of fibrous tissue and is partially covered by the epidermis which is always discontinuous at the tip in detached seeds. Remains of a slender vascular strand are usually present in a canal-like cavity which pierces the fibrous tissue (Text-fig. 2 F–H) and may represent the destroyed phloem. The pedicels found attached to a few seeds consist of a cylinder of large-celled parenchyma (45 μ) in the centre of which is a space containing the xylem (Text-fig. 2 A). This parenchyma intergrades with the fibres of the seed, but the epidermis of the seed has not been traced into the pedicel. Abscission presumably occurred at the junction between the pedicel parenchyma and the fibres. The seeds appear to have been obliquely attached to their pedicels. In one example (α 24) the pedicel was observed to arise as a branch, 1 mm. long, arising from an axis which was traced for 2 mm. in either direction from the point of junction, but unfortunately the preservation was very poor.

e. The apical pole. The parenchymatous central column rests on the apex of the megaspore and is surrounded by the lagenostome; the whole protrudes from the lipped apex of the integument (Text-fig. 2 K). The parenchyma where it fits like a cap over the apical papilla of the megaspore consists of radially flattened cells (10–30 μ radially by 20–40 μ tangentially by 60–90 μ long) as compared with those of the column proper (10–15 μ in diameter by 80 μ long).

The lagenostome can always be demonstrated but its preservation is very imperfect even in the best specimens. Where seen standing free it is composed of a single layer of cells. These cells are probably epidermal in nature (Text-fig. 3 A). Their size apart from tangential diameter (30–40 μ) is uncertain but the one cell measured in length was 200 μ. Within the lips of the integument it is occasionally possible to demonstrate that the lagenostome is formed of a double layer of cells. On either side of the major plane the lagenostome is only included for about one-ninth of the seed length (Text-fig. 2 B); compared with about one-third in the major plane (Text-fig. 2 A and B).

In the reconstruction, the integument is represented as being lipped all the way round at the apex (Text-figs. 1 A and 2 K). The evidence for the continuity of the lip in the major plane comes mainly from the xylem traces which in any series of transverse sections always end before the lagenostome. Further, there is nothing in any of the sections to suggest the presence of apical processes. The lips are thought to have been fleshy and parenchymatous in the major plane as opposed to those in the minor plane which were wholly fibrous.

Microspores have only been found within two of the seeds. In both they occurred near the base of the annular cavity formed between the lagenostome and the central column (Plate, Fig. 2). They are round with levigate coats, but it has not been possible to demonstrate any haptotypic structure. The average diameter for the six spores measured was 36 μ with the range from 28 μ to 45 μ.

DIAGNOSIS

Eosperma gen. nov. Seed bilaterally symmetrical with an intimately fused nucellus and integument. Apex of integument lipped with a protruding lagenostome surrounding a central column of parenchyma. Integument composed of an epidermis, an outer fibrous tissue, and an inner parenchyma. Vascular system of two xylem traces which arise by the forking of a single strand at the base and which run round the seed in the principal plane.

TABLE I

Dimensions of Seed and Megaspore

				Average	Minimum	Maximum	Number of measurements
Seed	Height . .		α	3·34 mm.	3·09 mm.	3·90 mm.	5
			β	—	3·15 mm.	3·75 mm.	2
	Major diameter .		α	3·35 mm.	3·15 mm.	3·75 mm.	11
			β	—	3·06 mm.	3·66 mm.	2
	Minor diameter	At apex	α	1·23 mm.	1·02 mm.	1·50 mm.	6
			β	1·27 mm.	1·06 mm.	1·32 mm.	4
		At base	α	0·55 mm.	0·15 mm.	0·66 mm.	7
			β	—	—	0·60 mm.	1
	Elliptical pedicel diameter			—	0·25 mm.	0·50 mm.	1
Megaspore	Height . . .			2·48 mm.	1·90 mm.	2·96 mm.	16
	Major diameter . .			2·12 mm.	1·54 mm.	2·72 mm.	17
	Minor diameter .			0·70 mm.	0·60 mm.	0·96 mm.	20
	Height of apical papilla.			0·28 mm.	0·15 mm.	0·45 mm.	15
	Major diameter at base of apical papilla .			0·55 mm.	0·30 mm.	1·05 mm.	15
	Thickness of the membrane . . .			6·6 μ	4·5 μ	8·0 μ	20

Megaspore discoid with a prominent apical papilla. Megaspore membrane intimately invested externally with a cuticular network of cellular dimensions.

Eosperma oxroadense sp. nov. Seed with a rounded outline in the major plane, and cuneiform in the minor plane; outline in transverse section a flattened ellipse; seed attached to the pedicel by means of a small basal papilla. Specific dimensions given in Table 1 above:

forma α with a smooth epidermis.
forma β with unicellular epidermal hairs.

The type material in the form of cut blocks, and prepared slides are in the Gordon collection, Geology Department, King's College, London. The slide numbers are: petrological sections, 1994, 1995, 2036, 2037, 2041, 2042; peel sections, 2113 to 2189. The type specimen is seed α 19, slide nos. 2144 to 2148.

The locality is Oxroad Bay just south of Tantallion Castle in East Lothian (National Grid reference: NT 597847).

The horizon is the Green Ash within the Cemenstone Group (Upper Tournaisian) of the Calciferous Sandstone Series, Lower Carboniferous.

The generic name is from the Greek ἠως dawn and σπέρμα a seed. The specific epithet refers to the type locality.

DISCUSSION

The inclusion of *Eosperma* in the order Lagenostomales may not at first sight appear justified. However, the points of structural similarity do appear to outweigh the single point of difference, namely, that of symmetry. For example, there is a general similarity between the 'pollen-catching apparatus' in *Eosperma* and that in such a seed as *Lagenostoma.* I was particularly impressed by the similarity between the central column in *Eosperma* and the tissue filling the beak (or salpinx) leading to the pollen chamber (or lagenostome) in *Calymmatotheca kidstoni* Calder in a new slide shown to me by Mr. A. G. Long. The apical bulge of the megaspore can be compared with that in *Physostoma.*

Conostoma platyspermum Graham (1934) which comes from the Upper Pennsylvanian (? Stephanian) of Illinois presents the most marked example of bilateral symmetry in the Lagenostomales. This seed which is very much younger than *Eosperma* differs in having radially elongated hypodermal stone cells, as is typical of all other seeds in this order so far described. Even more distinctive, however, is the small included lagenostome typical of the genus *Conostoma.*

Samariopsis scotica Calder (1938) is the only petrified platyspermic seed described from Lower Carboniferous rocks. This seed is also from Cementstone strata and comes from a locality only twenty-five miles from Oxroad Bay. *S. scotica* is, however, quite distinctive, in its shape, its histology, and especially in the form of its lagenostome (pollen chamber).

The megaspores of *Eosperma* obtained by maceration bear close comparison with those described by Arnold (1948) from the Lower Pennsylvanian of Michigan. In particular the large cuticularized reticulum on the outer surface of the *Eosperma* megaspores shows a marked similarity to that which surrounds the megaspore of *Spermatites reticulatus* Arnold. Graham's fig. 36 (1934) of the tapetum of *Conostoma platyspermum* is also of interest in this respect as are Thomson's (1905) comments on the suberized tapetum which surrounds the megaspore in living Cycads. I have further observed polygonal markings on the surface of the megaspore membrane in *Encephalartos.* Similar but smaller markings on the megaspore membrane of *Callitris glauca* are figured by Lurzer (1956, fig. 8) who notes their endospermal origin. It is probable that the possession of a cuticularized 'tapetum' was a relatively common feature of palaeozoic Gymnosperms. A contrast to living Gymnosperms is to be found in the fine structure of the megaspore membrane. Hoskins and Cross (1946) described the megaspore membrane in *Pachytesta vera* as a fibrous reticulum and if this latter structure is anything like that of *Cystosporites* figured by Schopf (1938, pl. 8, figs. 3 and 6), it is very different from *Eosperma*, where the fine structure is more like a sponge. The thickness of the megaspore membrane is a very variable character about which some information is now available. For the purpose of comparison this is given in the following table:

TABLE 2

Seed	Author and date	Megaspore membrane μ	Spore size mm.	Age*
Spermatites globosus	Arnold (1948)	12	3·2×3·0	Westphalian B
Stephanospermum elongatum	Hall (1954)	18–35	8×5	Westphalian D
S. stewarti	Hall (1954)	10	6×4†	Westphalian C
S. ovoides	Hall (1954)	20	4×3†	Westphalian D
Pachytesta vera	Hoskins and Cross (1946)	12–21	28×20	Westphalian C
Conostoma quadratum	Neely (1951)	5–7	2·8×0·9†	Stephanian
Taxospermum undulatum	Neely (1951)	9–11	3×2·3†	Westphalian D
Eosperma oxroadense		4·5–8	2·5×2·1	Tournaisian
Lagenostoma ovoides		4–9	3×2	Westphalian A
Sphaerostoma ovale		3–7	2·5×1·5	Visean

* Ages given on correlation with the European standard.

† Approximate dimensions obtained by measurements from published text-figures and plates.

The megaspores of free-sporing and seed-bearing lycopods have been increasingly studied in recent years. The dimensions of the megaspores from seed-bearing lycopods are generally comparable to those of the seed megaspores in the table. Seed compressions in shales do sometimes yield the megaspore and surrounding cuticles. I have further succeeded in obtaining the megaspore from the nutlike seed casts known as *Trigonocarpus parkinsoni* Brongniart. Dijkstra (1956, pl. 6, figs. 65, 66, and pl. 11, fig. 123) figures specimens suggestive of seed megaspores obtained by maceration of core samples. It is possible that a careful search of macerated coal will in future reveal further seed megaspores.

Little has been discovered about how the seeds may have been borne on the parent plant. That the seed stalks were probably part of a dichotomous branch system is suggested by the stalk of seed α 24. This together with the platyspermic nature of the seeds might suggest a remote similarity to a megasporophyll of *Cordaianthus pseudofluitans*. The structure, however, does not suggest a close relationship to the Cardiocarpales, where there is typically a nucellus more or less free from the integument.

Two specimens were discovered inside a cupule of the *Calathospermum* type. Though there is a superficial similarity in the histological preservation of the pedicel of *Eosperma* to that of the stalks in the cupule and though the hairs on the forma β are of comparable diameter to those on the outer lobes of the cupule no union has been observed. Professor Walton in his description of *Calathospermum scoticum* (1949) demonstrated the attachment of seeds of the *Salpingostoma* type and I now have evidence that the Oxroad Bay cupules were the parent bodies in which the seeds known as *Salpingostoma dasu* were borne.

Although it is possible that *Eosperma* seeds were borne within some kind of cupule as yet unknown, I favour the view that they were probably borne directly on the ultimate rachises of a frond or branch system. In this connexion one can compare them with the compression *Wardia fertilis* described by D. White (1905), from the Lower Pottsville of West Virginia. There, flattened rhomboidal seeds were found attached to the fronds of the *Aneimites* (*Adiantites*) type.

It is of further interest to note that a major constituent of the Oxroad Bay flora is *Tetrastichia bupatides.* Gordon suggested that this plant bore the compression fronds known as *Sphenopteris affinis* L. & H. sp. His reasons were the bulbous base to the petiole, the sclerotic inner cortical nests in the basal region, and the 'sparganum' outer cortex of the rest of the petiole. He suggested that his failure to detect any pinnae on the petiole below the dichotomy in the one specimen where there was connexion from the stem to the dichotomy was perhaps exceptional. Similar exceptions were claimed for *S. affinis* where a pair of sub-opposite pinnae are usually present on the petiole below the dichotomy. My own observations have suggested that the absence of pinnae on the petiole of *Tetrastichia* was a more general state of affairs. If this is correct, a correlation with the frond-genera *Adiantites*, *Aneimites*, *Diplotmema*, or *Spathulopteris* would appear more natural.

SUMMARY

1. A new Lower Carboniferous seed *Eosperma oxroadense* is described. It is platyspermic yet its pollen-catching apparatus shows more similarity to the Lagenostomales than to any other group. The megaspore investment is not clearly differentiated into integument and nucellus.

2. The megaspore membrane, which is well preserved, is apparently exceptional in showing a sponge-like fine structure. The outer surface of the megaspore bears polygonal markings. Similar markings, where known on the megaspores of fossil and living seeds, are noted for comparison.

3. The possibility of finding seed megaspores in coal macerations is suggested and a table giving the sizes of described seed megaspores is included.

4. The seed appears to have been borne terminally on some kind of branching axis. The identity of the parent plant is unknown.

ACKNOWLEDGEMENTS

I am greatly indebted to Professor J. H. Taylor for the loan of slides and cut blocks from the Gordon collection. I have great pleasure in expressing my thanks for the advice and encouragement received from Professor C. T. Ingold, Professor T. M. Harris, Professor J. Walton, Mr. W. G. Chaloner, and Mr. A. G. Long; also to Dr. K. L. Alvin for help and criticism; Mr. McGregor for photographic assistance and the Governors of Birkbeck College for a Research Studentship which has enabled me to carry out this work.

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PLATE

Eosperma oxroadense gen. et sp. nov.

Fig. 1. Median apical minor plane section, showing the central cone of parenchyma. Seed β 2, G.C. 2156 (peel 4). ×80.

Fig. 2. Microspores at base of lagenostome. Seed β 10, G.C. 2134 (peel 5). ×500.

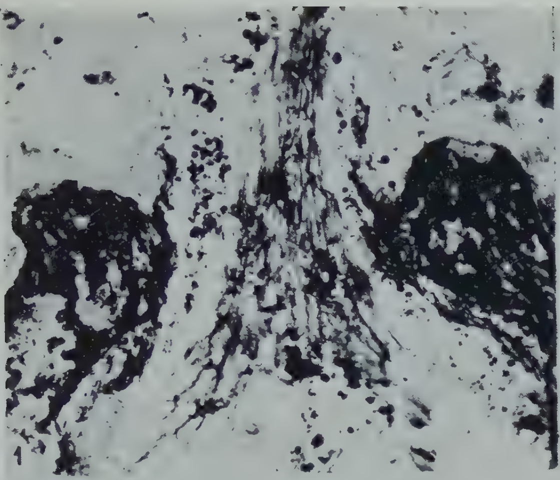
Fig. 3. Slightly oblique major plane section, passing medianly through the base and tangentially through the lagenostome. Rock section, G.C. 2041. ×20.

Fig. 4. Transverse section of megaspore membrane (8 μ) showing three layers. *is* = inner spongy layer, *os* = outer spongy layer, *h* = middle homogenous layer. G.C. 2189. ×2,000.

Fig. 5. Single layer of megaspore membrane showing fine microscopic structure. G.C. 2180. ×2,000.

Fig. 6. Transverse section just below the apex showing the lagenostome, fibrous tissue, and xylem traces. Seed β 5, G.C. 2171 (peel 4). ×60.

Fig. 7. Median major plane section passing through the basal papilla and showing the fusion of the xylem traces. Seed α 9, G.C. 2155 (peel 1). ×40.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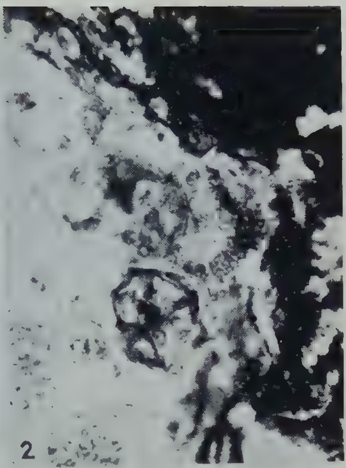
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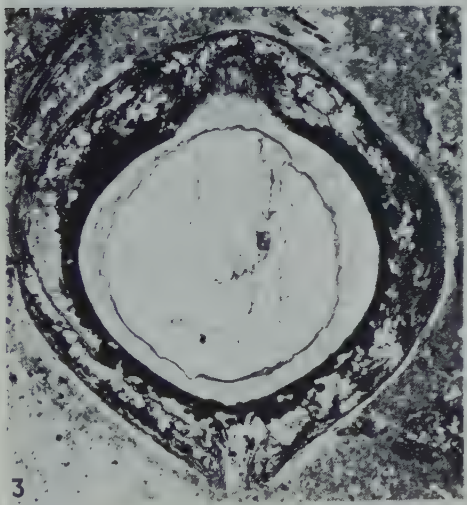
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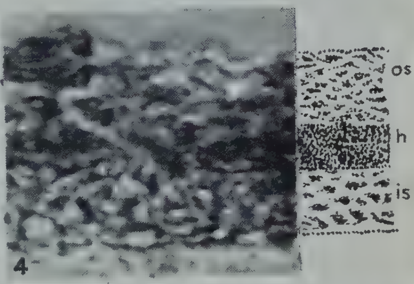
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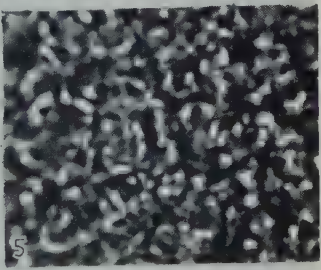
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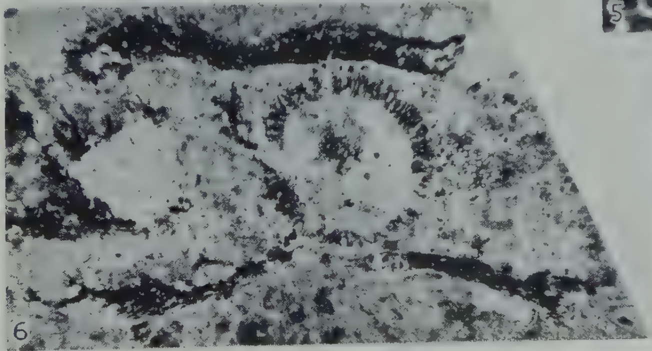
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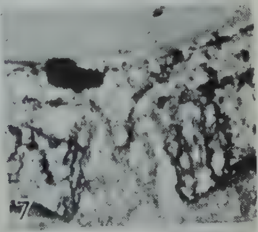
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P. D. W. BARNARD

Spore Discharge in the Basidiomycetes

I. Periodicity of Spore Discharge by *Trametes gibbosa* Fr.

BY

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With two Plates and three Figures in the Text

ABSTRACT

Variations in the rates of spore discharge from this fungus have been examined in controlled laboratory conditions, using apparatus designed to make a continuous record of the numbers of spores discharged by a strip of hymenium measuring approximately 4·5 cm. × 0·1 cm. These records have been extended by a direct microscopic study of the numbers of spores falling through a small area below the hymenium during successive short periods.

Analysis of the continuous record has shown that at any one time the rate of spore discharge is not uniform over the whole hymenium, and that at any one site it is not constant. The variation in the rate of discharge in any one site is rhythmical, with intervals of the order of 30 to 90 minutes between maxima. The small linear areas of hymenium which have been examined have shown nearly the same rhythm-overall, but the phase of this rhythm has differed at different sites in the area.

INTRODUCTION

DIURNAL rhythm in sporulation has been reported in the Phycomycetes and in the Ascomycetes. The work on the Phycomycetes has been reviewed and extended by Schmidle (1951) and by Uebelmesser (1954), that in the Ascomycetes by Buller (1909), Ingold (1928, 1933, 1946), Ingold and Cox (1955), Ingold and Dring (1957), and Yarwood (1936, 1937, 1941). In the Basidiomycetes von Schrenk (1900) stated that he saw the spores of *Polyporus schweinitzii* 'coming off at intervals', and Banker (1910) reported similar irregularities in spore liberation from *Steccherinum septentrionale*. Buller (1909, 1922, 1924, 1931, 1935) reviewed the other scanty early work on the rates of spore discharge and spore fall in the Basidiomycetes in general. He described the different types of movement of zones of basidiospore discharge across the hymenium of the genera resembling *Panaeolus*, and of the genera resembling *Coprinus*. From observations on a variety of genera he concluded, however, that except for these movements there is no periodic variation in spore discharge rate in the group, and that Banker's observations were 'probably erroneous'.

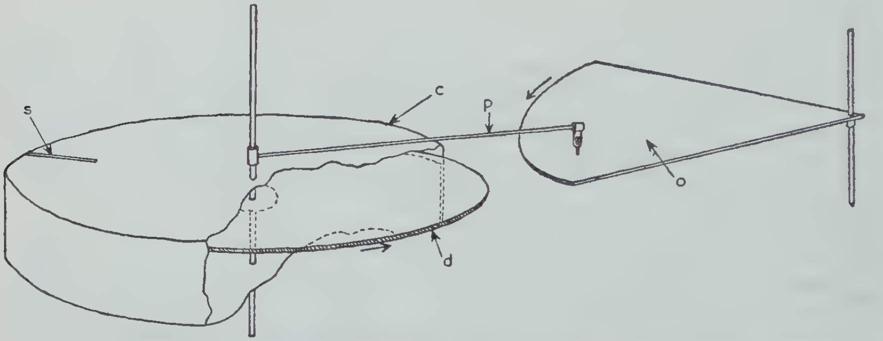
In the autumn of 1955 some irregularity was noted in the density of spore deposits on glass coverslips held for short consecutive periods below small areas of hymenium of *Trametes gibbosa* in the field. The following report deals with an examination of the process in this species in laboratory conditions,

[Annals of Botany, N.S. Vol. 23, No. 90, 1959.]

using an apparatus designed to record variations in rate of spore discharge from small areas of hymenium.

MATERIALS AND METHODS

The principle of the apparatus used to collect spore deposits is shown in Text-fig. 1. It consisted of a horizontal glass disc (*d*) 26 cm. in diameter which was rotated continuously at uniform speed close below the top of a cylindrical aluminium case (*c*). A radial slit (*s*) in the top of the case was 0·1 cm. wide and 4·5 cm. long. The disc was rotated either by an electrically-driven or by a spring-driven clock mechanism. Where necessary additional gearing was introduced to give a rotation such that the spores falling through the slit produced a visible deposit on the disc. In one experiment the uniformity of speeds of rotation of the two types of mechanism when assembled in the apparatus in the incubator was compared by attaching the pen (*p*) to the electrical mechanism and recording its movements on chart (*o*), which was simultaneously rotated at a different speed on a different axis by the spring-driven mechanism. The rotation of the spring-driven mechanism was also examined in laboratory conditions by recording the line traced by a similar pen as it traversed a chart which was moved continuously between rollers on a line radial to the disc by a separate electric motor. In both these tests a smooth line was drawn when the two components were rotating at constant rates, and any experimental change in these rates was recorded by an irregularity in the line. The rotation of the spring-driven mechanism was also examined by marking the position of a pointer attached to its spindle every 2 minutes during 2 hours' rotation.



TEXT-FIG. 1. Diagram of the apparatus used for the collation of spore deposits, with part of the case cut away to show the assembly of the glass disc.

The sporophores of *Trametes* were collected from sycamore stumps in Helensburgh (V.C. 99) in October–December 1956, from Raasay (V.C. 104) in July 1957, and Bearsden (V.C. 99) in November 1957. Blocks of wood about 15 cm. × 15 cm. × 15 cm. in size, each bearing a single sporophore, were cut from the colonies and kept on damp sand in a Glasgow greenhouse till required. Immediately before each test the wood block was thoroughly soaked with tap water and a pad of wet cotton-wool was placed on the upper surface of the sporophore. It was then secured above the case of the apparatus shown in Text-fig. 1, with the slit (*s*) running approximately radially to the curved edge of the sporophore. The sporophore was orientated so that the hymenial pores were approximately vertical and their lower ends were as close as possible above the slit. As the under surface of the sporophore in this species is normally inclined at an angle of up to 10° from the horizontal the distance between the top surface of the slit and the bottom of the pores varied from approximately 0·2 cm. to 1·0 cm. The whole apparatus was held in the dark in an incubator at 20° C.

In the earlier experiments a thermometer beside the slit was read on opening the incubator. In later experiments a thermograph sensitive to changes of the order of 0·3° C. per hour was run in the incubator. In one experiment a thermistor was placed 1 cm. from the slit on the upper surface of the case and coupled to a continuously recording device which was sensitive to temperature changes of more than 0·05° C. in 5 minutes. In one other experiment an old radon source was exposed a few mm. above the disc surface immediately before it passed below the slit. The measured emission from this source was about 1000 β particles per second.

The experiment was repeated seven times with sporophores from Helensburgh, five times with sporophores from Raasay, and once with a sporophore from Bearsden. The electric clock mechanism was used for two experiments with the Helensburgh material, the spring-driven clock mechanism was used for the remainder. One sporophore from Helensburgh was examined three times, and one from Raasay twice by the spring-driven mechanism, and one from Helensburgh was examined by both mechanisms. The remaining experiments were each on different sporophores. The same water-jacketed incubator was used in all experiments, but this was provided with additional water cooling and moved to a different building for the tests of the Raasay and Bearsden sporophores.

The study of spore deposit was supplemented by direct observation of the movement of spores in still air below the hymenium, using an apparatus similar to that devised by Buller (loc. cit.). This consisted of two 2 cm. square glass coverslips held approximately 0·1 cm. apart by a plasticine seal on three sides. This cell was held vertically with its narrow open side approximately 0·2 cm. from the lower end of the hymenial pores of an actively discharging sporophore. A parallel beam of light was directed at an angle of approximately 70° on to the back of the coverslips, and a horizontal microscope was focused upon the enclosed air space a few mm. below the top. The assembly was enclosed within a damp cardboard container to prevent extraneous draughts and to avoid the drying of the sporophore during the experiment. The microscope eyepiece was positioned to protrude through a hole in the side of the container. The spores falling in the focal plane of the microscope appeared as bright reflecting spots, and variation in their relative numbers was recorded by determining the time taken for 20 spores to cross a line 1 mm. wide in the centre of the field. The rate of spore fall in these conditions was determined at regular intervals by recording the time taken for a single spore to fall through 2·5 mm. in the field. With rare exceptions all the spores in a field at any time retained their relative position throughout the visible period of spore fall, so that the single reading taken correctly represented the whole population visible at the time.

RESULTS

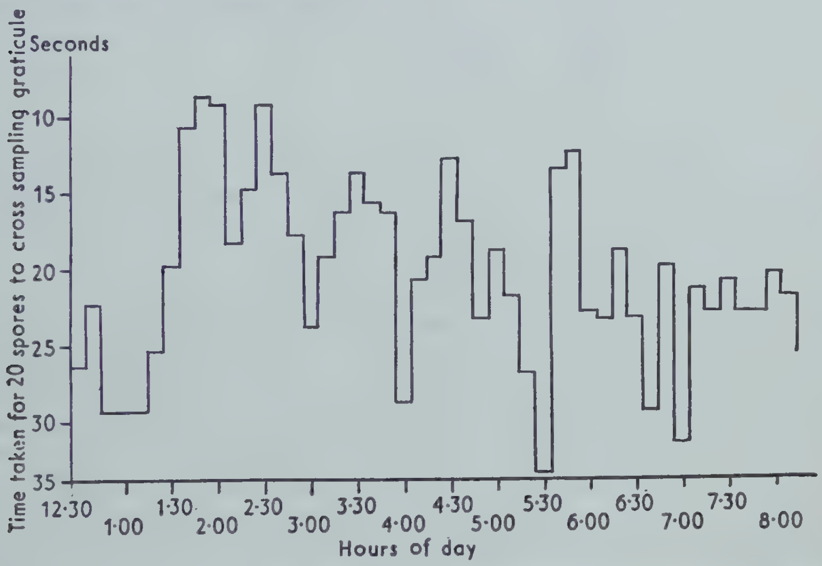
The pattern of spores deposited on a disc in a typical experiment is shown at Plate 1B. This disc was rotated by the electric clock motor at a rate of 5° per hour, and spore deposits were first visible on a slide covering the slit 18 hours after the sporophore had been placed in the apparatus. The slit was exposed 1 hour after this time and spores were allowed to fall through on to the rotating disc for a further 22 hours. The times are shown during which the incubator was opened for inspection or for minor adjustment of the apparatus, and those during which uniform rotation was recorded. The thermometer on the surface of the case approximately 3 cm. from the slit showed variations in temperature within the range of 19° C. to 21° C. during the periods of inspection.

There is a regular radial series of white areas formed on the disc in the undisturbed period from 6½ hours to 20 hours from the beginning of the experiment. These are each the result of an increase in the number of spores deposited on it in the period of approximately 25 minutes during which that area was moving immediately below the slit. The two main series of these deposits lie on alternate radii, and each is approximately half the length of the slit. A thinner deposit of spores is present between these dense areas. Both series of dense deposits recur at regular intervals of the order of 35 minutes during the undisturbed period, and traces of the pattern are visible in the deposits during the disturbed conditions at the beginning and the end of the experiment. In some areas indistinctly defined dense deposits lie obliquely to the disc radius.

The details of the patterns of deposits differed in all experiments, though there was much similarity in those formed in the different experiments with the same sporophore. All patterns showed a rhythmical variation, however, with dense deposits recurring at intervals of the order of 30 to 90 minutes, and some lines of dense deposit lying obliquely to the radius of the disc. Plate 2B illustrates part of the deposit on a disc rotated by the spring-driven mechanism below another Helensburgh sporophore, and the temperature changes in the immediate neighbourhood of the hymenium during this period. Recurring patterns of oblique lines are visible within the dense deposits.

Similar recurring patterns within the dense deposits are visible in Plate 1A and Plate 2A, but in these the periods of maximum deposit are more sharply defined and they are separated by a longer time interval. The deposits in Plate 1A were obtained from a Bearsden sporophore using the spring-driven mechanism, and the plate was exposed to β-irradiation during approximately half the time of the experiment. Each dense area is made up of two overlapping oblique zones of deposits which show only slight change in alignment throughout the experiment, and the pattern is similar in both the irradiated and the unirradiated portions of the disc.

In Plate 2A, which was obtained from a Helensburgh sporophore using the electrically driven apparatus, the orientation of the dense areas changes during the experiment. Initially they overlap obliquely, subsequently the outer part, nearest the circumference, becomes radial while the inner part remains oblique. Later the outer part disappears and the inner part becomes radial and finally the inner part also disappears.



TEXT-FIG. 2. Graph of result of a direct count of the numbers of spores falling from the hymenium of a sporophore of *Trametes gibbosa* held in a constant environment.

The results of a direct count of the numbers of spores falling below the hymenium in controlled conditions are shown in Text-fig. 2. The variation is highly significant (variance ratio = 8·1 for $n_1 = 46$, $n_2 = 423$), and the least significant difference between any two points plotted on the graph is 7·6. A similar significant variation was found in an examination of a sporophore from Raasay by the same method.

The mean rate of spore fall seen by direct observation during the experiment from which Text-fig. 2 was obtained was 1·8 cm. per minute, with limits of 1·2 and 2·2 cm. per minute. In a subsequent experiment using another sporophore from Helensburgh the mean rate was 2·5 cm. per minute, and a few spores fell at up to 3·75 cm. per minute.

DISCUSSION

In these experiments some variation in the rate of spore discharge has been seen by taking samples from sporophores in the field, and by direct observation of spore fall in the laboratory. Rhythmical variation has, however, been demonstrated only by mechanical sampling in a laboratory incubator. Any interpretation of the results must therefore consider the possibility of their having been affected by undetected irregularities in the rate of rotation of the disc, or of the physical condition of the collecting surface, or by an undetected rhythmic change in the air currents passing between the hymenium and the collecting surface. Any of these factors could cause a pattern in a deposit of spores which had been shed at uniform rate by an hymenium.

The following discussion therefore deals with two points:

(*a*) The possibilities of undetected irregularity in the apparatus or of fluctuating air currents having had a direct effect on the spore pattern.

(*b*) The relationship between the patterns and any variation of spore discharge rate from the hymenium.

(*a*) *Irregularities in apparatus and in air currents*

(i) *Irregularity in rate of rotation of the disc*: Similar patterns were obtained using apparatus controlled by two different clock mechanisms, each of which appeared to be in good order. Clock mechanisms were chosen because they are designed to prevent irregularities in rotation. The amount of play in the final drive was in all cases less than the distance between the successive dense deposits in the patterns, and it is unlikely that any irregularity due to the taking up of such play would appear at regular intervals under a constant torque. Irregularities in the spore deposits developed during some of the periods in which the mechanical tests recorded a uniform rate of rotation. These tests were not carried out continuously during all experiments, however, as it was thought that, with a constant rate of vertical spore fall, irregularities in the rotation rate could give rise only to radially disposed irregularities in the deposit pattern, each of which would be the same length as the slit. No system could be visualized whereby irregular rotation alone could give rise to the complex series of oblique patterns seen in Plate 1A, and Plate 2A and B, or to the series of dense deposits on alternate radii which have formed below different parts of the slit in Plate 1B.

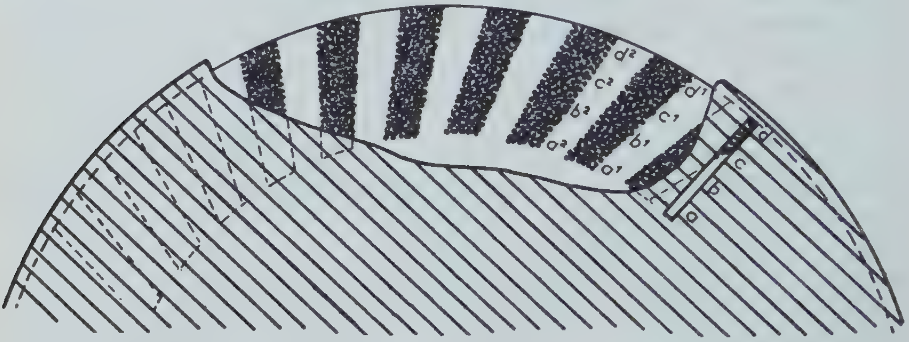
(ii) *Effect of possible irregular distribution of electrostatic charges on the adhesion of spores to the surface:* Fungal spores are known to bear charges, and variation in electrostatic charges between different areas of a collecting surface might therefore affect the pattern of spores deposited on it. Such variations are possible on a non-conducting surface such as the dry glass disc used in these experiments, and irradiation was used in one experiment to dissipate any such charges. It was not possible to calculate the precise conductivity induced in the air above the disc by this irradiation, but tests of the radon source using an electroscope indicated that this would have been sufficient to dissipate any charges likely to be present. The experiment recorded in Plate 1A shows that this irradiation had no significant effect on the deposit pattern.

(iii) *Air movements*: During this work Buller's descriptions of the turbulence in the spore cloud caused by slight air currents below the hymenium have frequently been confirmed by direct observation. The regularity of the patterns produced on the slowly rotating discs suggests, however, that the air is little disturbed by their use, and in still air the slit sampling device ensures that the pattern is a record of the spore deposit from a constant small area of the hymenium. The irregularity in the patterns which are exactly synchronous with known disturbances in the environment suggests that the conditions are relatively undisturbed during most of the experiments, though it is unlikely that the air is sufficiently still for exactly vertical spore fall. Though such air currents must have had some effect on the deposits, it is difficult to visualize any condition which would give rise to the very localized and clearly defined currents which would have to recur at regular intervals in the same relation to each other to produce the complex patterns recorded in these experiments. The microscopic study of falling spores gave further information of the possible effects of air currents. In the still conditions in which the results given in Text-fig. 2 were obtained all the spores were falling vertically, and mostly at the same rate. Their relative positions were maintained so regularly that small groups which appeared as showers in limited areas could be identified throughout their descent across the field. Air currents produced by slight known interruptions disturbed this arrangement immediately, causing changes in, and in some cases the complete reversal, of the direction of movement. The very striking difference in the appearance of the spore cloud in the two conditions discounts the possibility that the variations shown in Text-fig. 2 were due to fluctuating air currents. The cause of the appearance of the 'showers' of spores in parts of the field had not yet been examined, but their size and arrangement suggest that they may be groups discharged from a single pore.

(*b*) *The relationships between the patterns and variation in spore discharge rates*

The presence of more than one series of discrete groups of spores on different radii in some patterns, and of oblique zones of dense deposits in other ones, gives information of the way in which the rate of spore discharge varies with time between adjacent small areas of the hymenium. The angle between radii passing through discrete groups of spores, or through the inner and outer ends of the oblique zones, is commonly equivalent to the angle through which the disc has rotated in 10 minutes or more. If maximum periods of spore discharge were simultaneous over the whole of the hymenium above the slit and the spores were falling at constant rate vertically, this displacement of the deposits could only have been produced by differences in the distance through which the spores fell. These distances would have to be discontinuous to produce discrete groups of spores, or successive to produce oblique zones. The lower surface of the sporophores used showed no such discontinuous differences in height above the disc, however, and the total depth of the pores plus the distance from the pores to the disc surface did not exceed 3·5 cm. in any case. The slowest rates of spore fall observed in the experiments was 1·2 cm. per minute, hence the variation in time of fall might have contributed to an angular displacement of the pattern equivalent to the distance moved by a point on the disc in 3½ minutes. This would apply in extreme cases only, as most spores were observed to fall at about 1 to 2 cm. per minute. This factor could therefore not be responsible for the deflections equivalent to the distance moved during 10 minutes.

From these analyses it is therefore inferred that the patterns of the deposits are the result of a rhythmic variation in the rate of spore discharge from parts of the hymenium. The uniform radial deposits are thought to be due to synchronous development of high discharge rates over the whole hymenium above the slit, and it is appreciated that this could be incited by some undetected rhythmical change in the environment. The times of the slight changes in temperature recorded in Plate 2B do not coincide with the change in the pattern, however, and it is difficult to visualize a system of delayed reaction to such stimuli, by limited areas of hymenium, which could result in the formation of the more complex patterns. The formation of discrete series of groups on different radii of the form seen in Plate 1B could be due to the rhythm of attainment of maximum rates in the hymenium above the inner end of the slit to be out of phase with that in the hymenium above the outer end. The oblique deposits are thought to be the result of successive differences in the time of attainment of maximum rates in adjacent areas of hymenium above the slit. The result of such a system is illustrated in Text-fig. 3.



TEXT-FIG. 3. Diagram to illustrate the pattern of spore deposit which would be produced on a uniformly rotating disc if there was a successive difference in time of sporulation in small areas of hymenium above the slit. Deposits a_1–d_1, a_2–d_2, &c., represent the spores deposited by areas of the hymenium *a–d* at the positions shown above the slit, during uniform anti-clockwise rotation of the disc. At the time of the drawing area *c–d* is just finishing discharge.

This technique of sampling from a small linear area has shown that in many cases the 'direction of movement' and interval between zones of maximum discharge remain constant for a period, resulting in the deposition of an orderly series of overlapping spore groups at regular intervals. In other cases, however, the zones have changed in alignment or have been superseded by other zones with a similar rhythm of development which are 'moving' in different directions. The technique gives no information, however, of the total amount of hymenium which is sporulating at maximum rate at any time.

Nor does it show whether the recorded deposits below the slit are those from a series of small discrete areas or whether they represent transects across or along the line of broad 'waves' of sporulation crossing the whole hymenium. In the latter case the appearance of radial deposits on the plates may be due to the waves passing the slit across its length, the oblique deposits being those produced by movement along its length as in Text-fig. 3. Any complete analysis of the pattern must also allow for the fact that the deposits are an accumulation in one plane of spores discharged from an hymenium lying in three planes above it. It is suggested that Buller failed to see this variation because it was masked by the complexity of rhythm phases in the large areas he sampled.

Further work is now being carried out to study the movement and extent of these zones in relation to environmental or internal stimuli, and to examine the distribution of the phenomenon in other Hymenomycetes.

ACKNOWLEDGEMENTS

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EXPLANATION OF PLATES

Each radial division of the scale around each figure indicates the angle through which the disc rotated in 1 hour.

PLATE 1

A. Photograph of spores deposited by a sporophore from Bearsden during an experiment in which the disc was exposed to irradiation for the period shown.

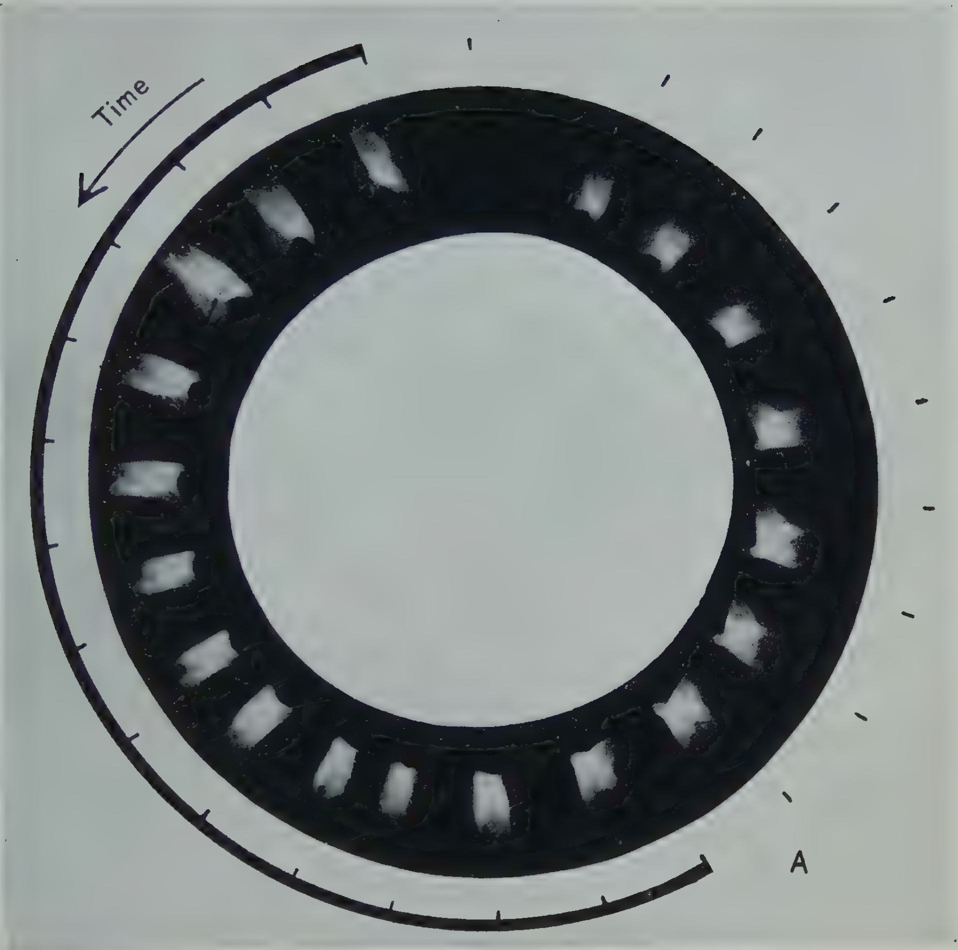
B. Photograph of the spores deposited by a sporophore from Helensburgh.

PLATE 2

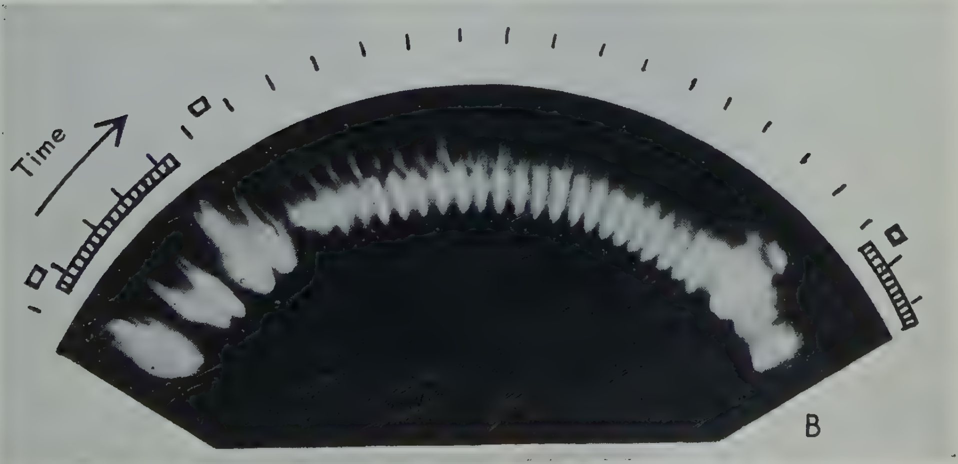
A. Photograph of the spores deposited by a sporophore from Helensburgh.

Note. The marks at the edge of the figure at the base are caused by the supporting device used during photography.

B. Part of photograph of spores deposited by a sporophore from Helensburgh.



▬ Time during which the disc was exposed to a radon source



□ Times during which the incubator was opened for inspection

▥ Times during which the rate of rotation was recorded

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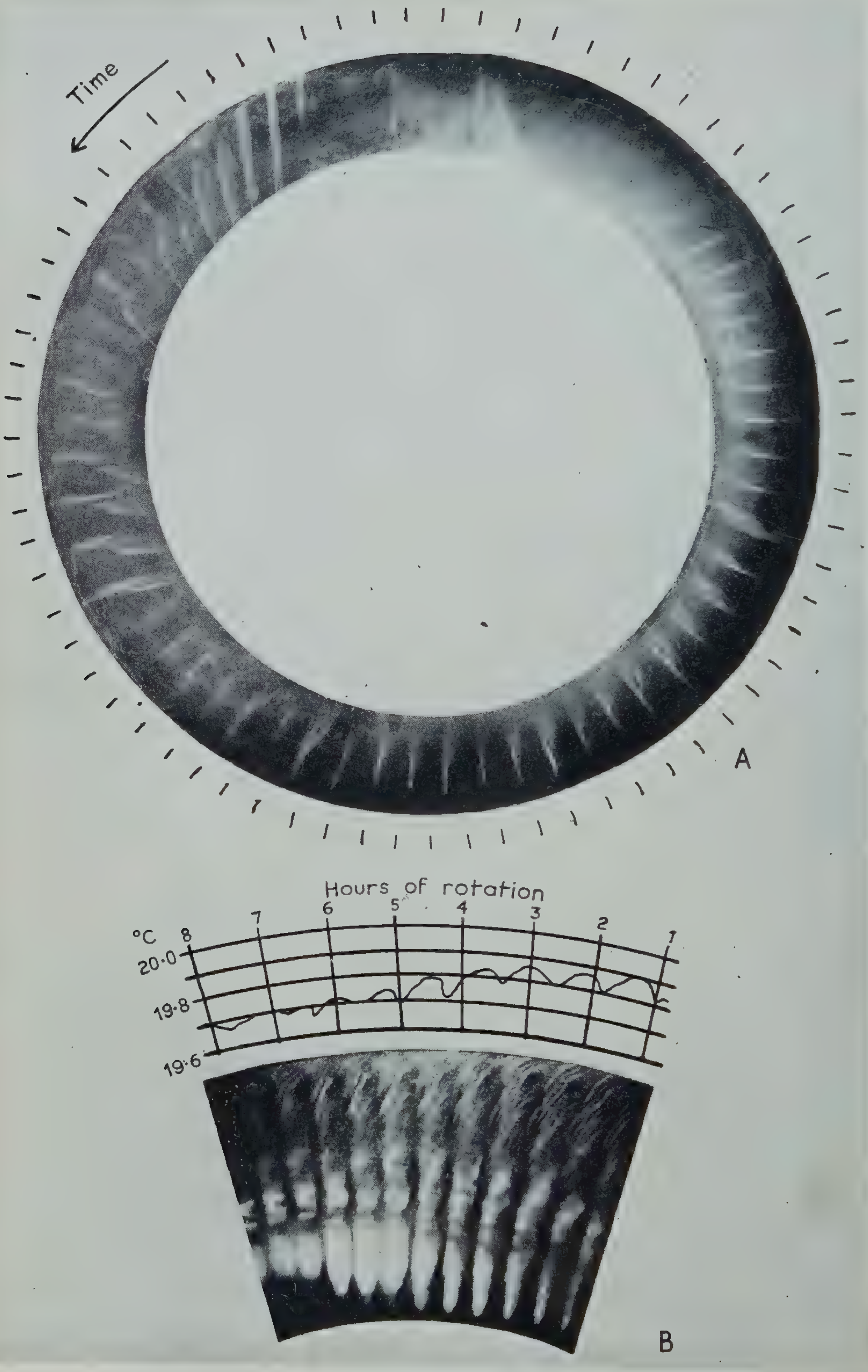

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Natural and Induced Rooting of the Stem Apex in *Rubus*

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With seven Figures in the Text

ABSTRACT

European *Rubi* of the subgenus *Eubatus* spread vegetatively by the rooting of stem apices at the end of the growing season. The rooting reaction involves a cycle of changes beginning at the time of the equinox with an acceleration of extension growth and the assumption of positive geotropism. Entering the ground, the stem apex forms a rooting boss, from which a short negatively geotropic shoot terminating in a resting bud arises. This resting bud develops in the subsequent spring to establish an independent daughter plant. A sequence of events resembling the natural rooting reaction can be induced in midsummer by darkening the apical 25 cm. of vegetative stem apices whilst still attached to the parent plant. Roots are formed within 3 weeks of darkening, and a dormant bud after 4 weeks. It seems probable that the natural rooting reaction is a response to shortening day length in the late summer.

INTRODUCTION

IT is characteristic of most of the European brambles of the subgenus *Eubatus* Focke that at the end of the growing season, during the period of the ripening of the fruit, some stem tips become positively geotropic and, entering the ground, form a well-developed adventitious root system. By this reaction in successive years the plants are enabled to form the dense spreading entanglements so typical of the arching stemmed *Rubi*. The earliest comment on the rooting of stem apices in brambles appears to have been by Pliny, and many of the batologists of the last century called attention to the phenomenon (Weihe and Nees, 1822–7; Bell Salter, 1845; Babington, 1869; Lees, 1884). Similar rooting reactions occur in scrambling and thicket forming shrubs and herbs of a number of other genera, e.g. *Forsythia suspensa* Vahl., *Jasminum nudiflorum* Lindl., *Malus sieversii* (Ldb.) M. Roem., *Vinca* spp., and *Calystegia* spp., always as an adaptation facilitating rapid vegetative spread and reproduction. The *Eubati*, however, appear to be unique in that it is the extreme stem apex which bears roots, after becoming positively geotropic, whereas in the other plants just listed roots are formed on any of the more distal parts of the stem which happen to come into contact with the ground. The rooting reaction in *Rubus* was first described as occurring specifically in the autumn by G. de St. Pierre (1875). Francis Darwin (1880) assumed that the reaction was a response to gravity, but later became doubtful of this interpretation after observing bramble stem tips rooting both upwards and downwards on a hillside; he did not, however, suggest an alternative explanation.

[Annals of Botany, N.S. Vol. 23, No. 90, 1959.]

In the course of a cytological survey of British *Rubi* (Heslop-Harrison, 1953) use was made of the rooting capacity of stem apices to obtain root tips, and preliminary experiments on certain physiological aspects of the response were carried out. The technique used to force rooting was to enclose stem apices excised at a certain stage in dark boxes with moist moss. Resende (1956) caused *Rubus* apices to root by artificially darkening them whilst still attached to the plant; he attributed the spontaneous 'tip rooting' in nature to the fact that the soil, or wall crevices, provided the necessary darkness. The present paper reports a study of the natural rooting reaction and the cycle of events leading up to it, and provides a comparison with the rooting behaviour of stem apices induced by artificial darkening. For the purposes of the study attention has been confined exclusively to *R. ulmifolius* Schott, but what has been found for this species is probably also referable to all other tip-rooting brambles.

Anatomical and physiological aspects of the rooting reaction will be considered in a further communication.

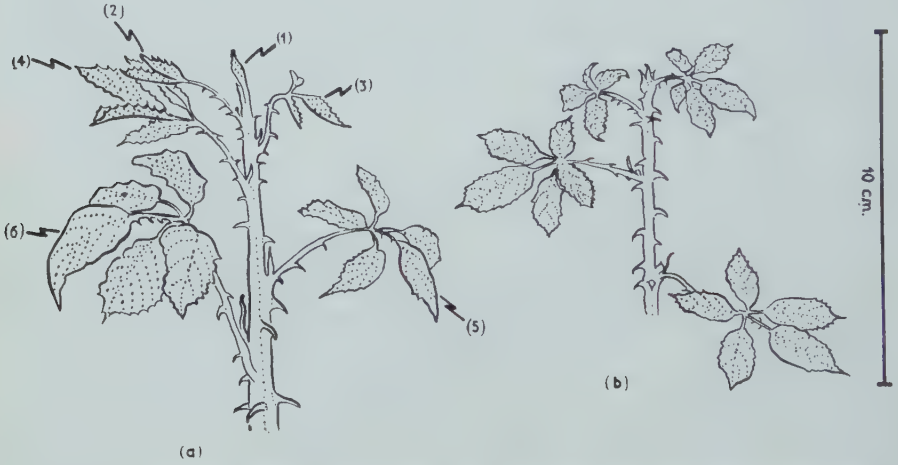

FIG. 1. (*a*) A long-day vegetative stem apex. (*b*) A dormant apex produced in short days.

GROWTH HABIT

At the commencement of spring growth there are two classes of stems which will develop in the current season, the primocanes and the floricanes (Bailey, 1941). Primocanes are the first year, ordinarily sterile, main stems; the floricanes are stems, 2 years old or more, which bear lateral inflorescences either directly or upon their younger lateral branches.

(*a*) *The primocane.* This develops from the base of an established plant as a strong, erect, succulent shoot. The phyllotaxis is $\frac{2}{5}$ and the leaves, in uniform light, become orientated evenly round the stem. The lower part of a primocane remains permanently rounded in cross section in *R. ulmifolius*, and bears rather slender, relatively weak, scattered prickles. The upper part becomes prominently five-sided and bears very strong, broad-based, subequal, hooked prickles on the angles. A mature stem is at least 1 cm. in diameter, with prominent ridges and slightly concave faces. In full sunlight it becomes dark red and pruinose, but in shade the anthocyanin does not develop. The extending apex is a pale shining green, and for a distance of 10–15 cm. is hardly lignified (Text-fig. 1*a*). Towards the end of the summer, when the stem has reached a height of a metre or more, the apex becomes plagiotropic, and the portion of the stem differentiating later is arcuate. It is rarely possible, however, to observe totally undamaged shoots, because the commonest habitat of *R. ulmifolius* in this country is in hedgebacks where extending shoots are exposed to cutting, grazing and wind damage (Text-fig. 2*a*). At the end of the growing season an undamaged primary shoot normally develops a dormant terminal bud of small, tightly packed, grey tomentose leaves, none over 3·5 mm. long, and the stem behind the terminal bud becomes tough and woody (Text-fig. 1*b*). Occasionally, however, the

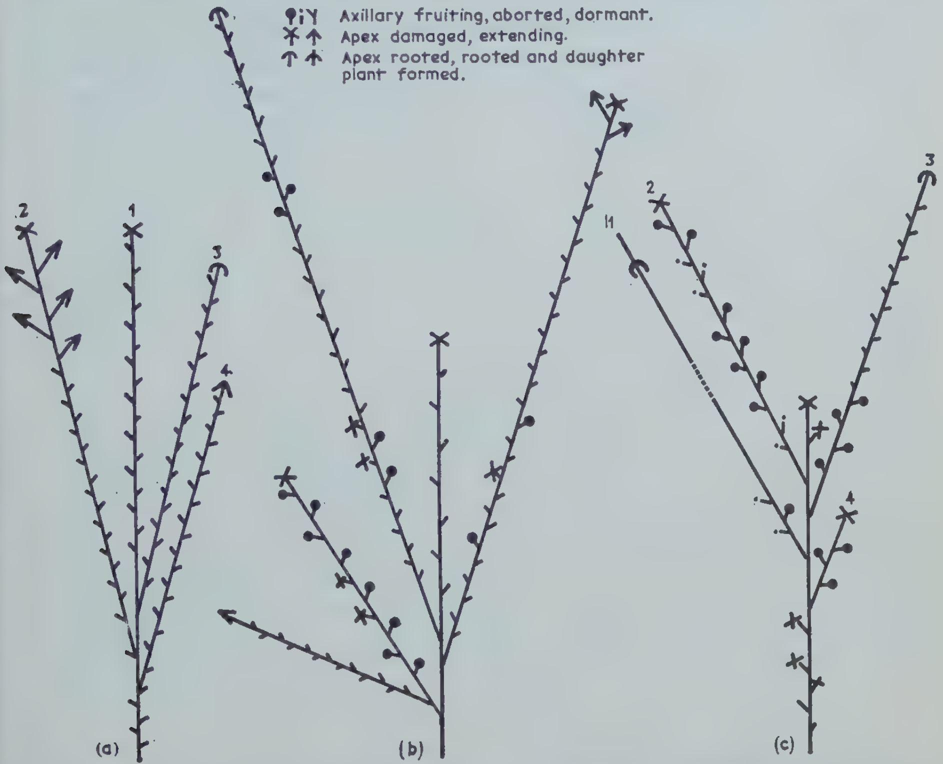
Axillary fruiting, aborted, dormant.
Apex damaged, extending.
Apex rooted, rooted and daughter plant formed.
2
1
3
4
(a)
(b)
2
1
3
4
(c)

FIG. 2. Diagrams of typical late season stems of *R. ulmifolius*. The state of the buds is as indicated in the key. (*a*) A primocane (stem of the current season's growth). **2**, **3**, and **4** are lateral branches produced after the destruction of the terminal bud of **1**; (*b*) a 2-year-old floricane whose terminal bud was destroyed during its first season of growth; (*c*) a senescent floricane, over 3 years old. The lateral branch **1** is 3 years old and rooted apically at the end of its second season of growth; in its third season a daughter plant was established from the rooted apex and death of the parent stem followed.

apex of a primocane may respond to the shortening days of autumn by rooting in the same manner as the vegetative laterals of the floricanes, described below.

(*b*) *The floricane.* The fate of most of the terminal and axillary buds along the primocane is already determined at the end of the first growing season. In the following spring the stem toughens and thickens somewhat, and occasionally some of its first-year leaves survive into this second year. The buds, already for the most part predetermined, mostly develop as (1) flowering branches or (2) vegetative laterals, although a certain proportion remains dormant. (1) An axillary flowering branch consists of three main zones.

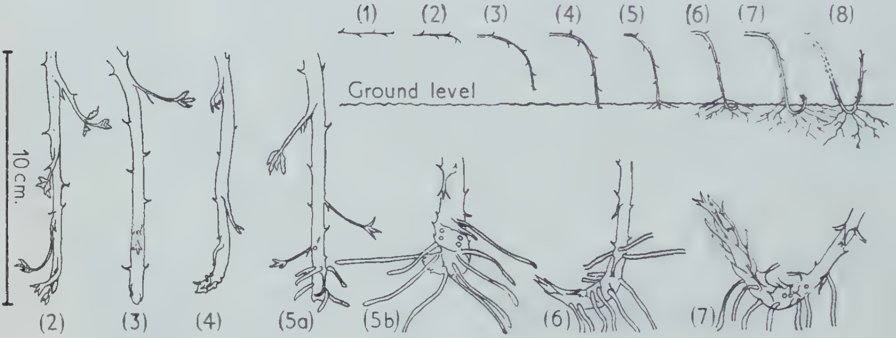

Fig. 3. Stages in the sequence of growth leading to apical root formation at the time of the autumn equinox. (1) Plagiotropic stem apex in long days; (2), (3) in short days the stem becomes positively geotropic, active stem elongation occurs, and the leaves become progressively smaller and more scale-like; (4) on reaching the ground apical extension almost ceases and root tubercles develop; (5), (6) the apical boss swells, the primary roots elongate and secondary roots develop; (7) in front of the rooting boss the apex becomes negatively geotropic and resumption of extension growth leads to the formation of a J-shaped shoot; (8) the establishment of a daughter plant in the following spring.

Below: Autumn-induced morphological changes in stem apices. In (6) and (7) the roots have been trimmed to show the appearance of the main axis.

Basally there is a group of three to four small ternate leaves grouped closely together; centrally the main axis bears five to ten simple or ternate leaves, and apically there are ten to fifteen flowering nodes, each bearing a group of three to five flowers in a cymose arrangement, the whole shoot terminating in a flower. After fruiting in September these shoots die and are shed and that portion of a floricane which has borne heavily fruiting axillaries often dies back also. (2) The vegetative lateral branches do not differ greatly in morphological features from the primocane described above, but they are usually less robust and, from the commencement of growth, are plagiotropic. In the shortening days of autumn they either respond by rooting apically in the manner described below, or become dormant. When an axis has rooted apically and established a daughter plant, it normally dies back slowly from the rooted region. Any uncommitted dormant buds on the lower parts of the stem may, however, be released from inhibition and give rise to fresh vegetative shoots. The lower parts of a floricane bearing such shoots may therefore be much more than two years old (Text-fig. 2 *c*). Text-fig. 2 *b* shows the type of extensive growth that frequently occurs when axillary buds are released from inhibition by damage to the leader.

(*c*) *The rooting of the stem apex.* By early September, apices of floricanes and their vegetative laterals become totally dormant or enter into a period of relatively rapid growth involving an ordered cycle of geotropic reactions and a series of well-marked morphological events which includes the production of adventitious roots. For the purpose of description this growth sequence has been divided into eight arbitrary stages (Text-fig. 3).

Stage 1. As a standard of comparison the morphological features typical of summer growth in long days may be taken. The extending stem tapers apically; it is plagiotropic and the phyllotaxis is $\frac{2}{5}$, but, by torsions arising in the petioles, the leaf laminae often become orientated horizontally. The terminal bud differentiates foliage leaves actively. The young leaf laminae are at first plicate, and take as long as three weeks to develop, so that only leaves some twelve nodes behind the apex are mature. Such a mature leaf is quinate-digitate with short, hooked prickles clothing the petiole, petiolules, and mid-ribs. At the base of the petiole is a pair of filiform stipules. The lamina is coriaceous in *R. ulmifolius*, a dark, rich shining green above and white tomentose below. The terminal leaflet is oblong-cuneate with a pronounced acuminate apex. The leaf margin is often curved downwards and is irregularly serratedentate. The stem characteristics correspond to those of the later-formed primocane described above.

Stage 2. This is the stage reached as the autumn equinox approaches. It is marked by a change in geotropic response of the stem apex, which becomes directed towards the ground. Morphologically the change is detectable in the less rapid expansion of the young leaf laminae.

Stage 3. This stage covers the interval between the onset of positive geotropism and the time of contact of the stem apex with the ground during late September or early October. As much as 2 metre of stem may be produced during this period of downward growth. The apex becomes blunt, not tapering, although it is still as active in differentiating nodes. Internode expansion is as pronounced as during the period of summer growth, if not more so, but leaves and superficial stem characters no longer mature. The portion differentiating during this period remains green permanently, without pruina or anthocyanin development; it is slender, whip-like and rounded in cross section, and retains its succulence for longer, while the prickles become smaller and weaker. The leaves, node by node, tend more and more to retain their juvenile character; there is less extension of the petioles, and the laminae, which at first unfold but barely expand, finally remain permanently plicate. The later-formed leaves are trifid, the central point representing the whole of the leaf lamina, and the two lateral points the stipules. A nodal sequence of thirteen such reduced leaves, which finally remain appressed to the stem, is illustrated in Text-fig. 4 *a*. The sequence of leaf and internode lengths along a typical stem is shown in Text-fig. 5 *a*.

Stage 4. As the stem apex comes into contact with the soil, or litter on the soil surface, and penetrates it, internodal extension slows down abruptly although nodal differentiation continues as rapidly as before. The result is the formation of a compacted series of nodes. In this region the stem becomes chlorotic and small tubercles become associated with the nodal scales.

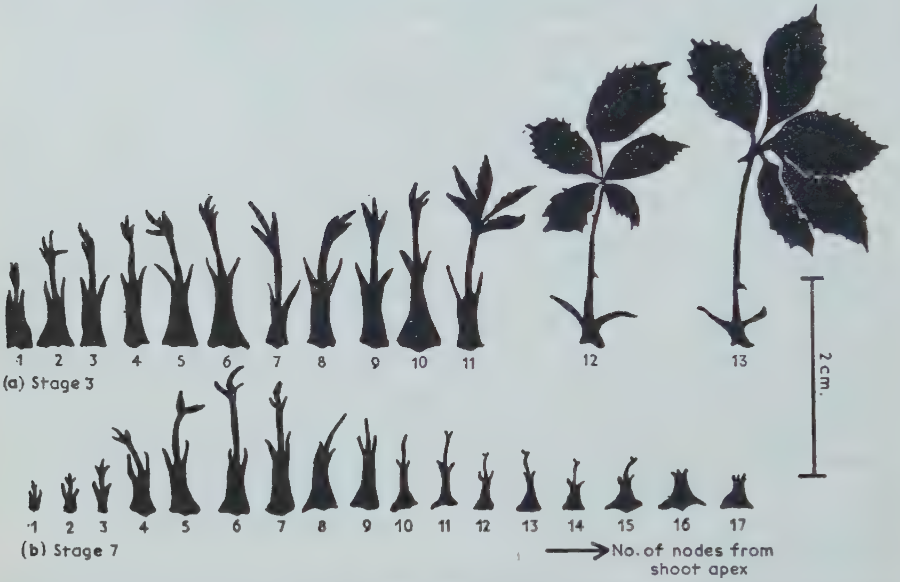

FIG. 4. Typical leaf or scale-leaf sequences from a positively geotropic Stage 3 shoot and a rooted Stage 7 shoot (see text and Text-fig. 3).

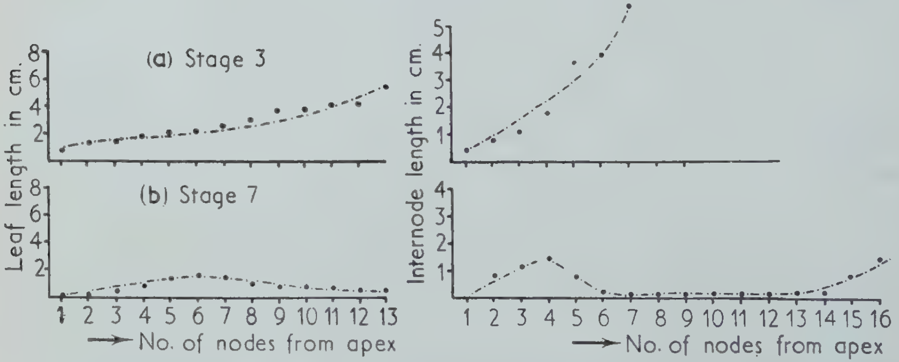

FIG. 5. Graphs of the sequences of leaf and internode lengths in naturally induced Stage 3 (means of 5 shoots) and Stage 7 shoots (single shoot).

Stage 5. This stage is marked by the rapid expansion of the tubercles as adventitious roots. The stem apex now becomes embedded in the soil, partly due to its own active penetration and partly by the pulling action of the developing roots. The first nodal scale to be associated with root formation does not differ greatly from those formed in the later part of Stage 4, but those differentiated when the apex is below ground are smaller and typically consist of little more than a contracted petiole base with very vestigial stipules and a lamina primordium. Often there are three roots associated with a single scale, one on either side and one directly behind it. Where roots are being actively differentiated, however, from five to eight may develop per scale, a pair on either side, and one, or a group of up to four, lying behind. Rather rarely anomalous groups may develop which do not appear to have any direct association with adjacent scales.

Stage 6. By the time the primary roots reach a length of 5 cm. or more, the axis has become swollen and tough and forms a pronounced boss of from six to twelve compacted nodes, usually devoid of prickles. The compacted region is rarely longer than 2·5 cm. The primary roots may grow to a length of 15–20 cm. and from them secondaries develop. At the apex of the stem, now beyond the region of the rooting boss, more nodes are differentiated. The growing point, having penetrated 2·5–5 cm. below ground level, completes its cycle of geotropic response and turns sharply upwards.

Stage 7. Beyond the region of the boss no more roots are formed. The new nodes being differentiated are separated by progressively longer internodes and, furthermore, become clothed by progressively longer scales as the soil surface is approached (Text-fig. 5). This sudden reversal in geotropic response follows the cessation of root formation and is accompanied by a return to internodal extension and an increase in size and development of the nodal appendages. A sequence of scales taken through the region of a rooting boss of ten nodes and up a J-shaped shoot of seven nodes is given in Text-fig. 4*b*. The growing point of the stem is now protected by bud scales and is at, or just below, ground level; it is at this stage, reached usually between the first and third weeks of November, that growth ceases until the following spring.

Stage 8. After the spring equinox a daughter plant is established by the extension growth of the J-shaped shoot. The stem union between daughter and parent plant dies back, tissue degeneration commencing behind the region of the boss and proceeding towards the parent plant; the polarity of the old stem is never reversed. Very often the oldest part of this stem continues to survive, producing panicles or new vegetative shoots from previously uncommitted axillary or serial buds.

EXPERIMENTAL INDUCTION OF THE ROOTING REACTION

In the second week of July, twelve actively growing apices from primocanes and vegetative laterals from floricanes from large, well established bushes of *R. ulmifolius* were enclosed in 60 cm. long, light-tight bags of black fabric. A total of about 25 cm. of stem was enclosed, including the youngest six to eight expanding leaves, the remaining 35 cm. of the bag being left empty to allow for subsequent extension growth. Equivalent shoots were marked as controls and left in natural long days throughout the period of the experiment. The appearance of a typical apex before treatment is shown in Text-fig. 1*a*:

the youngest leaf (1) is still appressed and encloses the terminal bud, the next three leaves (2–4) have plicate laminae, and the fifth is fully open, but still only about half the size of a fully grown leaf. The upper part of the stem is still green, tapers apically, and is succulent for the first 10–15 cm.

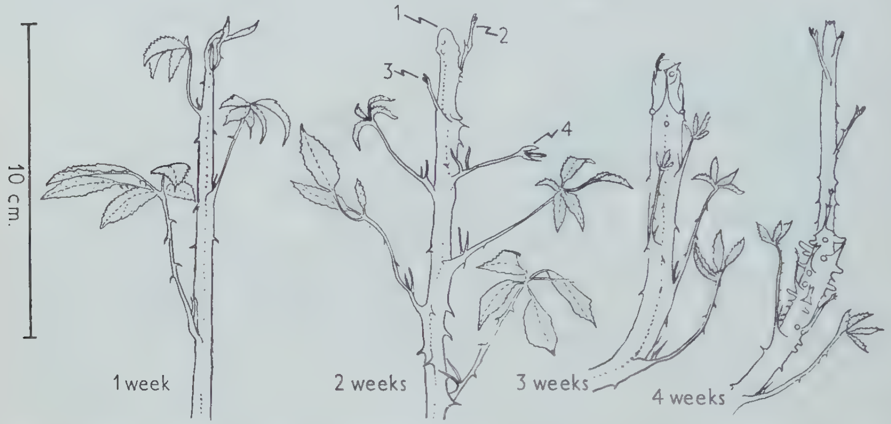

FIG. 6. Artificially induced rooting of *Rubus ulmifolius* stem apices by total darkness, 1–4 weeks after treatment.

The changes in the darkened apices, recorded at weekly intervals, are described below and illustrated in Text-figs. 6 and 7. After 4 weeks of darkness each apex had produced a rooting boss and, beyond this zone, a shoot terminating in a dormant vegetative bud. During the same period the control apices continued to make the normal extension growth characteristic of long days, and at the end of 4 weeks their apices still had the appearance of Text-fig. 1*a*. For the first fortnight of the experiment all the *R. ulmifolius* bushes under observation were without open flowers; during the third week most of the inflorescences were in full flower, and at the end of the fourth week only a few buds (*c.* 10 per cent.) remained to open.

The morphological and growth-rate changes in the darkened apices observed over the 4-week period were as follows:

(*a*) *7th day.* Growth, both in terms of nodal differentiation and internodal extension, showed an acceleration in the darkened apices compared with the controls during the first week (see below). There appeared to be little significant morphological change, but there was a very obvious colour alteration, the apical parts becoming markedly chlorotic.

(*b*) *14th day.* Rapid stem-extension growth continued, and during the first fortnight amounted to 10–15 cm., involving a total of 6–8 nodes. Over the same period of time the rate of growth of control apices was roughly half of this, and only 3–4 nodes were differentiated. The extreme stem apex had now thickened and become blunt, and other striking morphological changes were apparent, accompanied by complete chlorosis. The four upper nodes differentiated during the second week bore juvenile leaves appressed to the stem, very like the scale leaves formed during Stage 3 of the autumn-rooting reaction described above. Root primordia, appearing as hemispherical swellings, were associated with each of the three upper nodes (Text-fig. 6). The next three lower leaves had plicate laminae.

(*c*) *21st day*. Another five nodes were differentiated, each bearing roots up to 0·5 cm. long. The total of eight apical rooting nodes continued to thicken and toughen to form a boss essentially identical with that formed under natural short days. The internodes in this zone were compacted and the nodal appendages were small and scale-like. Behind this region the stem was more slender, the internodes were much longer, and the six leaves immediately behind the boss showed no further expansion of their plicate laminae.

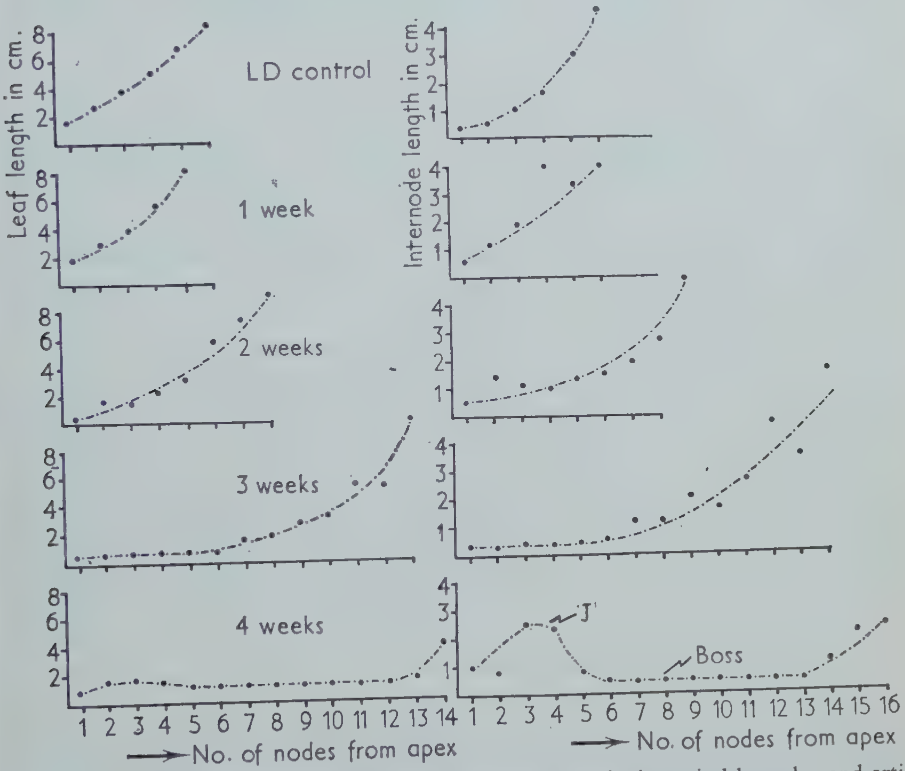

FIG. 7. Graphs of the sequences of leaf and internode lengths in typical long-day and artificially darkened shoots, 1–4 weeks after treatment.

(*d*) *28th day*. Beyond the rooting region, continued apical growth produced a shoot 5–7·5 cm. long of about five nodes with a maximum distance between the nodes of 3 cm.; along it the scale leaves increased in length and degree of development as successive nodes were differentiated up to the terminal bud. This shoot, the morphological equivalent of the J-shoot of Stage 7 in the natural short-day series, was, like it, markedly suffused with anthocyanin. Probably due to the fact that growth was restrained more or less to the horizontal within the dark bags, the striking change in geotropic response associated with the comparable stage in the natural tip-rooting reaction was not observed, the new stem beyond the boss in most cases continuing the line of the parent stem. At this stage the swollen nature of the boss region was very marked: the average diameters (five replicates) of the three main regions produced in direct response to total darkness—the succulent J-type shoot, the woody rooting boss, and the tough stem immediately behind the boss—being 5 mm., 13 mm., and 7 mm. respectively.

ANOMALOUS BEHAVIOUR CAUSED BY INJURY TO THE STEM APEX

In late summer and early autumn the usual effect of destruction of the terminal bud of a floricane is to release a large number of axillaries from inhibition and permit the outgrowth of a dozen or more lateral shoots. At the end of the season six or so of those near the site of injury commonly undergo the normal cycle of events leading to the production of adventitious roots. Although more slender than undamaged floricanes, these axillary shoots show considerable powers of extension, and a proportion may succeed in reaching the ground and taking root. Those which fail to do so become dormant, overwintering in a condition equivalent to Stage 3 described above. Occasionally small root tubercles may be formed at the apices of those which do not reach the ground before the onset of dormancy, but this condition has only been noted at the very end of the growing season in November. The extreme case of anomalous rooting caused by injury is where root tubercles are formed in association with the bud scales of axillary buds newly released from inhibition. Again, this is a rare end-of-season phenomenon, but that it should happen at all indicates that the outgrowth of roots is not dependent upon total darkness or the high humidity in litter or the upper layers of soil.

DISCUSSION

The above account indicates that the essential features of the natural end-of-season rooting reaction of the stem tips of *Rubus* can be induced by totally darkening the apical portion of vegetative shoots whilst they are still attached to plants growing in long days. The differences between the natural reaction occurring at the time of the autumn equinox and that which can be induced in midsummer in this manner are primarily in the relative duration of the phases, especially in the period of extension growth which precedes the formation of the apical rooting boss (Stage 3). Thus in the course of the natural reaction, as many as twenty nodes may be differentiated during this period with a long series of transitional leaves (Text-fig. 5 *a*), and the stem becomes attenuated and round in cross section. In the experimental series, no more than seven nodes were differentiated at this time, fewer transitional leaves were formed (Text-fig. 7, weeks 1–2), and the stems remained robust and angular up to the base of the rooting boss. It is significant that the duration of this extension phase in nature is fairly constant. The number of nodes differentiated is not related to the distance that the apex has to grow to reach the soil surface during the positively geotropic period; those which are close to the ground at the beginning of the extension growth do not root immediately upon reaching it, but grow along its surface until the standard number of nodes has been produced.

Thus total darkness has the effect of abbreviating a phase which in all reacting stems would normally extend over 3 or 4 weeks during a period when natural day length was gradually shortening to less than 11 hours. Whilst yet to be shown experimentally, it seems probable that a reaction more closely comparable to the natural one could be induced by simulating this gradual reduction in photoperiod over a comparable length of time. The period of positively geotropic extension growth preceding the formation of the rooting boss is evidently an essential part of the reaction as an adaptation facilitating vegetative spread in the arching-stemmed *Rubi*; without it the apices of aerial stems would not normally reach the ground at all. There may, however, be occasions when the penetration of apices into wall crevices would, as suggested by Resende (1956), provoke the rooting reaction, independently of prevailing day length.

In the region of the rooting boss itself, the number of nodes is much the same in naturally rooting apices as in those caused to root by total darkening. Normally by this stage the naturally rooting apex will in any case have entered the soil or litter on its surface and be experiencing darkness or light of low intensity. The number of roots produced is correlated with the number of nodes in the region of the boss, and is again much the same in apices induced to root by darkening as in those doing so naturally. Root extension was considerably restricted in the experimentally induced series, no doubt as a result of the dry atmosphere within the fabric bags.

The time of root initiation, both natural and experimentally induced, coincides with a period of minimal internode extension and marked thickening and toughening of the stem. Once the roots are formed at nine or so nodes, the stem reverts to a more normal pattern of growth, the internodes becoming longer and more slender and developing anthocyanin again. Progressively more expanded leaves are formed up to the time of production of the dormant apical bud (Text-figs. 5 *b* and 7 (4 weeks)), which, in naturally rooting apices, is developed after 5–6 cm. of growth. Somewhat more growth, 7–8 cm., was produced beyond the boss in the experimentally induced series, but ultimately a resting bud was formed, so completing the sequence of phases characteristic of the natural reaction. An inverse correlation of extension growth and root production seems to be a general characteristic of rooting stems; it has, for example, been demonstrated in isolated stem tips of *Asparagus* growing in culture in the presence of β-indolylacetic acid (Galston, 1948).

It is of particular interest that the rooting reaction can be induced by darkening the actively growing vegetative stem apices at a time when the remainder of the plant is receiving long days, and independently of its developmental state. Whilst this observation does not exclude the possibility that the leaves may participate in the natural reaction as photoperiodic receptors, it does indicate that the apices of stems which are not committed to the production of a flowering panicle are largely autonomous, responding directly to the light régime in which they are placed. It seems probable that the whole sequence of reactions, involving the change in geotropic response, the accelerated extension growth, boss formation, leaf reduction, rooting, and the ensuing dormancy may be related to light-governed variations in the auxin metabolism of the apex, and this aspect is currently under study.

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The Pruning Response of One-year Apple Trees

BY

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ABSTRACT

The response to pruning, in the simple woody system exemplified by 1-year-old rooted apple shoots, could be summarized by the equation

$$\frac{\text{Weight of stem of new shoots}}{\text{Increase in weight of old stem}} = 0{\cdot}6\,\frac{\text{Old stem length before pruning}}{\text{Old stem length after pruning}} - 0{\cdot}3$$

irrespective of the amount of growth or of whether all new shoots or only two were allowed to develop. In a group of smaller plants, the proportion of new stem increased slightly. The response could be largely attributed to a close relation between the total increment and the thickening of the old stem which was found to be

$$\frac{\text{Increment in old stem}}{\text{Length of old stem}} = 0{\cdot}0063 \text{ total increment} + 0{\cdot}039.$$

Increase in weight of the plants was favoured by treatments promoting early bud-break and rapid development of leaf area. It was not simply related to pruning.

INTRODUCTION

THE general growth responses of the fruit tree to dormant winter pruning are fairly well established (Gardner, Bradford, and Hooker, 1922) and are that

1. The individual shoots arising from a pruned branch are larger than those on an unpruned branch.
2. Despite the faster growth of the individual shoot the pruned tree does not equal the unpruned tree in size, at least until fruiting has checked the latter's growth.
3. For a given degree of pruning the size of the shoot growing from the pruned stem is positively correlated with the length of the stem before pruning (Lockard, 1956; Magness *et al.*, 1917).

There are other effects of great practical importance concerned with maturation of wood, inception and amount of blossom, position of blossom, fruit size and quality, but these may be considered side effects, secondary to the effect of pruning on new growth. Yet despite the wealth of detailed observations comparing the new shoot growth of pruned and unpruned trees, there is little data on the changes occurring in the other parts of the tree, particularly the roots. Knight (1934) described the responses of trees, 3 years old, to various degrees of pruning, and showed that pruned trees increased less in weight of roots and old stem than unpruned. He also showed that as pruning increased in severity, the new shoot increased in weight and length

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up to the point where the stem up to 2 years old was pruned off; beyond this there was a decrease in new shoot. Knight drew no final conclusions from his experiment, but Vyvyan, from an examination of Knight's data, and from general considerations, has put forward the view that growth responses to pruning tend to restore the balance between terminal and longitudinal meristems (i.e. between shoot apices and cambia) disturbed as a result of pruning (Vyvyan, in preparation). Knight's data fitted Vyvyan's interpretation of his hypothesis very well, and it was evident that further work was needed to test directly the influences of initial stem weight, surface, length, and number of shoots upon subsequent growth and its partitioning between the various parts of the plant. This paper reports one such experiment.

The minimum system on which tree-forming factors could operate required a woody stem whereon one or more cycles of annual thickening could occur, one or more buds capable of extension growth, and a root system, negligible in initial amount but capable of rapid development. These requirements were met by cuttings and young rooted woody shoots. It is true that information so obtained was to be related to the pruning response of older trees, but the balance of evidence seems to be that such trees are quantitatively, but not inherently, different from young trees; they are formed by the repetition of processes already instituted and occurring in young trees. These processes—stemming from the activity of apical meristems and longitudinal cambia—result in a progressively greater separation of leaves and roots, in an increasing length and area of the channels of supply and communication, and in an increasing number of terminations of such channels (leaves, stem tips, and root tips). There is some evidence that the buds themselves on older trees are not inherently different from those on young trees. Cannon (1940) found little difference in the growth resulting from bud-grafts taken from old and young apple trees, while in pears he found no greater difference than that between the top and bottom of a 1-year shoot.

The present experiment used young rooted shoots of an apple rootstock. These had been produced by earthing over young shoots the previous summer so that they rooted from their etiolated bases (Anon., 1956). By autumn these shoots were from 20 to 100 cm. long and from 3 to 12 mm. wide at the middle. By pruning shoots of various thicknesses, young plants covering a range of weights, lengths, and surface/mass ratios could be obtained. The topmost buds remaining on the more heavily pruned shoots would be older than those on the more lightly pruned and this possibly introduced an important growth factor. It is known that the apical and basal portions of one season's shoots differ in several physiological respects (Garner, *et al.*, 1955, Cannon, 1940).

MATERIAL AND METHOD

Rooted stoolbed shoots of the apple rootstock variety M.XXV were selected for length, diameter, and freedom from damage. Classes were selected with length and diameter respectively

(*a*) 90–95 cm. × 1·3–1·4 cm., symbol W (wide, i.e. thick)

(*b*) 75–80 cm. × 1·1–1·2 cm., symbol N (narrow, i.e. thin)
(*c*) less than 50 × 0·5–0·8 cm., symbol S (small).

Class (*c*) comprised material normally rejected for planting and was more variable.

The pruning treatments were:

Treatment	Code	Diameter Classes treated		
a. 5 cm. cut from the tip of the stem	T (tipped)	W	N	S
b. Half the *length* cut off	$\frac{1}{2}$	W	N	
c. Two-thirds of the length cut off	$\frac{2}{3}$	W	N	
d. W pruned equal in weight to $\frac{1}{2}$ Narrow	W = $\frac{1}{2}$N	W		
e. N pruned equal in weight to $\frac{2}{3}$ Wide	N = $\frac{2}{3}$W		N	

Tip pruning was used as a control rather than no-pruning so that all trees had some pruning cut. Shoots were weighed and measured before and after pruning, and a sample was dry-weighed. The standard error of each pruned group of 10 trees was 1·07 g.

Planting took place on November 28 and 29, 1955 in good conditions. All shoots were planted 10 cm. deep. There were 32 replicates in randomized blocks in a single long row. Spare plants of each treatment in 3-inch pots of field soil were plunged in the ground at the end of the row. Shortly after budbreak the plants in alternate blocks were disbudded to two growing buds per plant; symbols—R (restricted) vs. U (unrestricted). Buds emerging from below ground level were also removed when seen.

Harvesting by blocks began on 10 September and finished on 30 September; most leaves were still on the trees, but extension growth had ceased.

RESULTS

The results to be presented lead to two main conclusions: that the weight increase of the plants was determined largely by the rate at which they began growing, irrespective of their size; and that the distribution of growth was determined by the degree of pruning, irrespective of the increment.

The effect of the various treatments on the pattern of shoot growth is illustrated by the various sections of Fig. 1.

Development of the shoots. Despite the favourable planting conditions, budbreak was slow. The various stages of bud development were scored and the progress of development is shown in Fig. 1*a*. Treatment differences were apparent from budbreak; buds grew earlier on the more heavily pruned shoots and on the thinner shoots. These differences persisted throughout the growth of the plants, so that the longest shoots resulted from pruning off two-thirds and the shortest from tipping, though the effect of diameter in the end was slight.

Total stem. Although the number of buds breaking was proportional to the degree of pruning (Fig. 1*b*), most of them, especially the lower ones, failed to elongate, and remained as spurs with small clustered leaves. On the average the Unrestricted treatments bore 2·9 shoots longer than 5 cm., while the

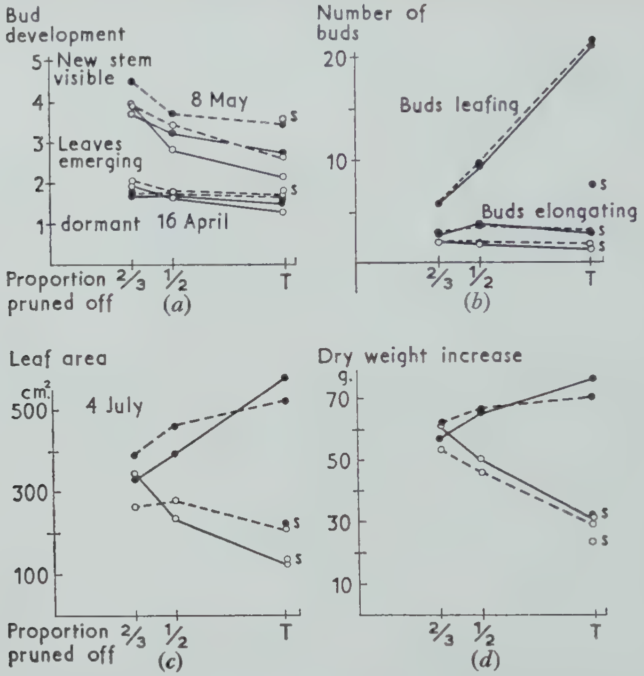

FIG. 1. *Effects of pruning on course of development:* (*a*) Bud development on two occasions in spring. (*b*) Number of buds breaking into leaf, and number of these buds subsequently elongating into shoots instead of remaining as short spurs. (*c*) Leaf area on 4 July. (*d*) Total increment in dry weight.

KEY: ● All buds allowed to develop (Unrestricted). ○ Top 2 buds only allowed to develop (Restricted). —— Wide class. – – – Narrow class. S Small class (only tipped).

Restricted treatments (2 shoots only) carried only 1·5 (tipped) and 2·0 (½ and ⅔ pruned). Consequently, total stem length, being the product of average length and number of stems, was considerably greater for the Unrestricted treatments than for the Restricted (Table 1).

TABLE 1

Total Stem Length (cm.) on 11 July and 11 September

	11 July			11 September		
Pruning	Tipped	½	⅔	Tipped	½	⅔
No. shoots						
R	19	33	47	50	87	106
U	46	54	51	116	133	123

Leaf area. The course of increase in leaf area (shown in Fig. 1*c*), like that of total shoot length, was the product of values for the individual shoot and the number of shoots. Differences between Restricted and Unrestricted trees were present almost from bud-break. Once stem elongation began, change in the magnitude of the differences between treatments was dependent on the relative numbers of shoots developing new leaves and, as has been shown, these differences also were established early on. There was thus a marked dependence of final size on early leaf area; treatment averages of leaf area on May 28 were significantly correlated with final leaf dry weight. The correlation was highly significant for Restricted treatments because most of the shoots grew long ($r = 0·86^{xx}$); the correlation was not significant for the Unrestricted plants because many of the shoots remained short ($r = 0·63$).

The effects of disbudding interacted with pruning. With heavy pruning, Restricted and Unrestricted trees had similar leaf areas throughout growth, but with lighter pruning total leaf area was less on Restricted plants and greater by about the same amount on the Unrestricted plants.

Dry weight. The dry weights, determined when the trees were harvested during late September, are given in Table 2. Fig. 1*d* shows how the variation in total increment resulted from the combined effects of pruning and disbudding. The weights clearly reflect the influence of early development and the data already presented have shown how these early differences have been built into the pattern of development to produce the final differences in increment. The original size of the plant (before pruning) had a marked effect on the relative growth-rate; *within* each pruning treatment, the planting weight of the smaller size class was multiplied more times than that of the larger. But if different sized trees were pruned to be equal in planting weight, they did not all grow to the same final weight. This point was tested specifically (Table 2, treatments 6 and 5, 7 and 8). The large shoots pruned severely (tr. 6) did not differ significantly from the smaller ones cut to half their length (tr. 5) but the large shoots ⅔ pruned away (tr. 8) were significantly lighter than the rather lightly pruned smaller shoots pruned to be equal to them in weight (tr. 7).

TABLE 2

Dry Weight of Plants. 15 September

Trt. No.	1	2	3	4	5	6	7	8	9	Sig. diff. ($P = 0·05$)
Size	W	N	S	W	N	W	N	W	N	
Pruning	T	T	T	½	½	=Tr.5	=Tr.8	⅔	⅔	
(a) Unrestricted, all shoots allowed to grow										
Total	97·8	84·1	34·9	80·8	81·7	75·2	90·0	69·1	70·4	17·1
Planting wt.[1]	22·2	14·3	3·8	16·3	12·2	11·9	12·7	12·6	8·7	—
Increment	75·6	69·8	31·1	64·5	69·5	63·3	77·3	56·5	61·7	—
(b) Restricted, 2 shoots allowed to grow										
Total	54·5	43·5	28·5	66·7	60·4	61·7	64·9	73·6	57·9	11·2
Planting wt.[1]	23·6	14·4	4·2	16·9	11·3	11·2	12·9	12·7	8·3	—
Increment	30·9	29·1	24·3	49·8	49·1	50·5	52·0	60·9	49·6	—

[1] Estimated from a sample.

Proportion of parts. Although the increment in weight was influenced by the number of shoots as much as by pruning, pruning was the over-riding factor determining the partitioning of that increment between new stem and old stem, leaf and root. To reduce the effect of different absolute increments, the weights of leaf, new stem, &c., have been expressed as percentages of the total increment for each tree. The average percentages are given in Table 3.

TABLE 3

Percentage Distribution of Increment

Treatment	1	2	3	4	5	6	7	8	9	Sig. diff. ($P = 0.05$)
Size	W	N	S	W	N	W	N	W	N	
Pruning	T	T	T	½	½	= 5	= 8	⅔	⅔	
Percentage leaf										
U	20	21	24	21	20	22	20	23	22	1·9
R	24	22	24	21	20	22	21	21	22	1·4
Percentage new stem										
U	15	16	23	29	28	36	26	35	35	2·6
R	15	14	20	29	29	37	29	37	34	2·9
Percentage old stem increment										
U	45	45	33	30	30	21	33	21	22	2·9
R	40	45	35	30	31	20	30	22	23	3·7
Percentage root										
U	20	18	20	20	22	21	21	21	21	2·0
R	21	19	21	20	20	21	20	20	21	1·8
Percentage leaf plus root										
U	40	39	44	41	42	43	41	44	43	2·5
R	45	41	45	41	40	43	41	41	43	2·6

The following conclusions may be drawn:

(i) The distribution was unaffected by the number of shoots (2 or Unrestricted).

(ii) The percentage of leaf plus root varied by not more than 6 per cent.; and therefore the percentage of total stem increment (new plus thickening of old stem) was also practically constant.

(iii) The percentage of new stem increased with the severity of pruning; the total thickening of the old stem correspondingly decreased.

(iv) Although there was little difference between the W (wide) and N (narrow) treatments, the S (small) one had a greater proportion of new stem.

It is known that the percentage distribution of increment may vary with the increment itself (Maggs, 1957), and so this had to be estimated before the effect of pruning on distribution of increment could be determined. The covariance between increment and proportions was analysed into the regressions due to treatment and the residual variation within treatments. These are set forth in Table 4. In this experiment the significant changes in proportion with change in increment are due almost entirely to the pruning and disbudding treatments. The variation in increment within treatments was not associated with any corresponding change in proportions, apart from percentage leaves, Unrestricted.

TABLE 4

Relation between Proportions of Parts and Increment in Weight

	Unrestricted (all shoots)	Restricted (2 shoots)
(*a*) *Variation due to treatments*		
$\frac{\text{Leaf}}{\text{Increment}}$	= 0·357 − 0·0022 increment***	Not significant
$\frac{\text{New stem}}{\text{Increment}}$	= 0·917 − 0·0097 increment*	= 0·0081 increment − 0·130**
$\frac{\text{Old stem inc.}}{\text{Increment}}$	= 0·0121 increment − 0·508*	= 0·721 − 0·0084 increment**
$\frac{\text{Root}}{\text{Increment}}$	Not significant	Not significant
(*b*) *Residual variation within treatments*		
$\frac{\text{Leaf}}{\text{Increment}}$	= 0·249 − 0·00056 increment***	Not significant
$\frac{\text{New stem}}{\text{Increment}}$	Not significant	Not significant
$\frac{\text{Old stem inc.}}{\text{Increment}}$	Not significant	Not significant
$\frac{\text{Root}}{\text{Increment}}$	Not significant	Not significant

It appears that there was a strict relation between increment, pruning and disbudding, and proportion; the proportions being determined by pruning, and the increment in turn determined by disbudding and proportions (Fig. 2). This may be made clearer by considering an example—the treatment means for percentage of new stem. With Restricted trees, the percentage of new stem increased with increasing increment, and with Unrestricted trees the percentage decreased. Moreover, for both the Restricted and Unrestricted moieties of a given pruning treatment, the percentages of new stem are nearly the same, e.g. Tr. 2, 16 and 14 per cent. (Table 3). Where the regression lines cross, for both moieties, increment as well as proportion are the same (Fig. 2, 35 per cent. new stem, 59 g. increment, two-thirds pruned off), but with less severe pruning, the proportion of new stem declines, and so the increments of the Restricted and Unrestricted moieties must increasingly diverge from one another.

The absence of overall changes in percentage of the parts, other than those due to pruning effects (apart from percentage of leaf on Unrestricted trees), is somewhat surprising in view of significant changes found in nearby comparable material (Maggs, 1958). However, this latter material was grown in pots, and made more growth; and it may be that it is only after a certain threshold level of growth has been reached that an excess is disproportionately distributed within treatments. The very small negative regression between percentage leaf and increment on Unrestricted plants may be ascribed in this experiment to a comparative inactivity of the spur leaves, suggested earlier in this paper. Where these comprised a large proportion of the total leafage, increments were relatively small, and the proportion of leaf high; but where most of the leafage consisted of shoot leaves on long shoots, increments were higher despite a lower proportion of leaves. Maggs (1957) found an analogous situation in vigorously growing shoots of *Prunus*, where, in the case of *P. spinosa*, the presence of large numbers of secondary and tertiary shoots (many of them spurs) was accompanied by a negative correlation between percentage of leaf and shoot weight, while in the case of *P. domestica*, where spur-like shoots were practically absent, there was no correlation.

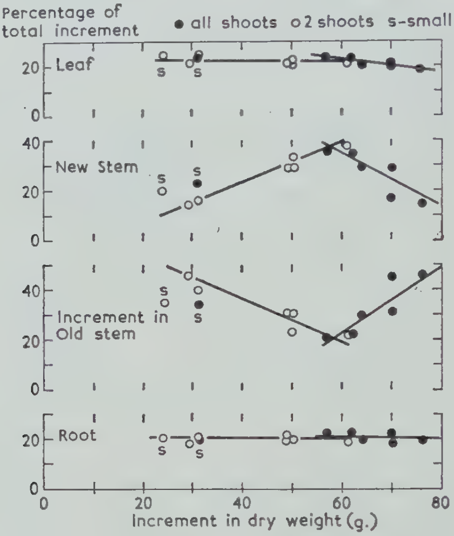

FIG. 2. Interaction of pruning, disbudding, and total increment. (Treatment means.) Open circles—restricted to 2 shoots; solid circles—unrestricted; S—small class.

PRUNING RESPONSE

It has already been shown (Table 3) that the percentage of new stem plus increment of old stem was nearly constant (average 57·6 per cent.); so, in considering the distribution of new materials as a response to pruning, we need only consider the relative amounts of new and old stem increment, and pruning response (R) may conveniently be measured as

$$R = \frac{\text{New stem weight}}{\text{Increment in old stem weight}}.$$

Considering the increment in weight of the old stem, in a set of variously pruned trees all making somewhat similar increments, it is to be expected that stems left unpruned and long will increase in weight more than the stems which have been pruned short. This is confirmed by the data of Table 3, which shows that only about one-fifth of the increment of the most heavily pruned trees was old stem thickening, as against nearly a half in the tipped trees. At the same time, the new stem is correspondingly relatively heavier so that the response will vary with the degree of pruning. *The actual relation between pruning and partition of increment is, however, quite precise.* If pruning is defined so as to increase with severity of pruning, as

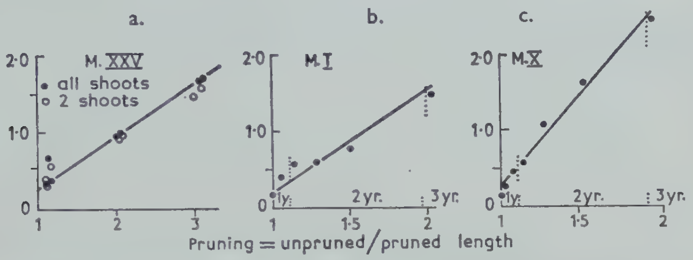

FIG. 3. The linear relation between pruning response and degree of pruning. The two graphs on the right are calculated from the data of Knight (1934), the youngest wood left after pruning amounts to 0·6 on the vertical scale.

$$P = \frac{\text{Initial length}}{\text{Length after pruning}} = \frac{L}{l}$$

it is found (Fig. 3*a*) that

$$R = 0{\cdot}6\,P - 0{\cdot}3. \tag{1}$$

In Fig. 3*a*, pruning is calculated on a length basis; an equally strict relation is found when calculated on a weight basis. The relation seems to be general, as similar, but not identical, equations were obtainable from the data of Knight which referred to scions 3 years old; these were calculated on a weight basis, and are also shown in Fig. 3 (*b* and *c*). Such a relation may be largely accounted for as follows. If the proportion of old stem increment is related to length as suggested, this can be written

$$\frac{\text{Old stem increment}}{\text{Total increment}} = kl. \tag{2}$$

It has been shown that the proportion of total stem is nearly constant (57·6 per cent.), so that

$$\frac{\text{New stem}}{\text{Total increment}} = 0{\cdot}576 - kl$$

and therefore

$$\frac{\text{New stem}}{\text{Old stem increment}} = \frac{0{\cdot}576 - kl}{kl} = \frac{0{\cdot}576}{kl} - 1. \tag{3}$$

l can be expressed in terms of degree of pruning, P, and the original length, L; $P = L/l$; and k can be determined by rewriting Eq. 2 as

$$\frac{\text{Old stem increment}}{l} = k \times \text{total increment.} \tag{4}$$

It may be noted that old stem increment per cm. length is equivalent to the cross-sectional area increase of the stem. This relation (4) was tested for each and all treatments, and it was found that a single regression line fitted the data for all treatments combined as efficiently as separate lines for each treatment. The pooled regression was

$$\frac{\text{Old stem increment}}{l} = 0{\cdot}0063 \times \text{total increment} + 0{\cdot}039 \tag{5}$$

and it accounted for 91 per cent. of the covariance. The terminal constant, 0·039, is small compared with the other terms of the equation and, to simplify the argument, will be ignored. If it is taken into account, it does not materially affect the conclusions. 0·0063 can therefore be put for k in Eq. (3). In substituting $1/l = P/L$, $1/L$ only varied between 0·011 and 0·012 (1/90·5 cm. and 1/84 cm.), so that Eq. 3 becomes

$$\frac{\text{New stem}}{\text{Old stem increment}} = \frac{0{\cdot}576 \times 0{\cdot}0115}{0{\cdot}0063} P - 1 = 1{\cdot}05\, P - 1. \tag{6}$$

Thus it is possible to account for a large part of the observed pruning response by what may be termed the geometry of the situation. Nevertheless, there is some discrepancy for which it has not so far been possible to account.

Discussion

This experiment has revealed two contrasting factors governing the amount and distribution of growth of the young tree. The amount of growth was largely determined by factors operating from bud break, and which affected the rate of development of the buds and number of buds breaking; the distribution of growth was in a fixed proportion between leaf-plus-root and stem tissues, and this latter was distributed between new stem and thickening of old stem in a proportion determined entirely by the pruning. It has not proved necessary to interpret the results of this experiment in terms of surface and mass, although such a relation is involved in the constancy of the ratio between increment and cross-sectional area increase of the old stem.

The differential effect of disbudding on the amount of increment under the various pruning treatments suggests an analogy with the decay of a signal along a transmission line; the longer the line, the weaker the signal received at the other end, and the more the sources (shoots) the stronger the signal; shoots along the old stem might be considered as boosters. If the signal is identified with growth-substance rather than nutrition or water, the preponderant effect on increment of the shoots over the roots is ascribable to the polarity of auxin transport, and a visual sign of propagation of the signal would be the downward growth of new conducting tissues in the spring following bud-break. This is readily demonstrated by bark stripping (Wareing, 1951; Priestley, 1935; Knight, 1927). However, although this may be plausible, it has not been possible to account quantitatively for the early differences between treatment by these arguments. The constancy of root percentages suggests that though the initiation of growth starts from the buds, it is subsequently limited by some function of the roots.

The strict relation found between increment, disbudding, and proportion of parts is at this stage difficult to account for. Since the slopes of the lines relating proportion and increment (Fig. 2) have different signs according to the disbudding, presumably there is some degree of disbudding at which the increment under various pruning treatments remains constant (the alternative, that there would be no pruning response, seems most unlikely). Experiments involving different degrees of disbudding are in hand and should give some evidence on this.

In the present experiment, given the regular distribution of increment, the crucial factor underlying the pruning response was the constancy of the cross-sectional area increase (stem increment/stem length) for a given *total* increment, irrespective of the actual diameter and length of the old stem. It may be noted that the cambium, situated as it is between the xylem and phloem, is favourably placed to respond to varying concentrations of substances in these tissues, provided they can diffuse therefrom. Definite grounds for considering such a suggestion must await information on the relation between the concentrations of transported substances and activity of the cambium and growth of the shoot.

The linear relation between total increment and increment per unit length of old stem has an interesting corollary. For trees of diameter D, growing to diameter $E = D+x$, the increment will be proportional to

$$E^2-D^2 = 2Dx+x^2.$$

Thus when the trees are small and x is of the order of D, the increment will vary with x more than D and correlations of increment with planting weight may be low. On the other hand if larger trees are considered, x^2 will be small compared with Dx and correlations of increment with size at the beginning of the experiment as well as with diameter increment are to be expected. Thus different relations may appear to hold according to tree size, both of which are compatible, within the latitude of a correlation, with the demonstrated relation between old stem area increment and total increment.

SUMMARY

1. The pruning responses in the growth of 1-year-old rooted shoots of the apple rootstock M.XXV are described. The shoots were selected to close limits within three size classes which, before pruning, weighed 43, 29, and 8 g. fresh weight. The shoots were tipped, or, in the two heaviest classes, pruned to $\frac{1}{2}$ or $\frac{1}{3}$ of their original length. When growth began, only two buds

were allowed to develop on half the trees (Restricted), on the remainder, all buds (Unrestricted).

2. Responses to pruning and disbudding were evident from bud-break and continued during growth. Heavily pruned, Restricted trees increased in total weight more than lightly pruned ones, while heavily pruned, Unrestricted trees increased less. The increment of Restricted trees averaged 49 g. and of Unrestricted trees 68 g. dry weight.

3. Twenty-two per cent. of the increment was leafage and 20 per cent. root. These percentages were nearly constant between and within treatments.

4. The residual 58 per cent. of the increment, comprising new stem and thickening of the old stem, was distributed between these two regions according to the pruning, irrespective of increment or disbudding, in accordance with the equation

$$\frac{\text{New stem}}{\text{Increment in old stem}} = 0{\cdot}6\frac{\text{Initial length}}{\text{Length after pruning}} - 0{\cdot}3$$

(99 per cent. of the covariance).

5. The increment in old stem per cm. length (equivalent to cross-sectional area increase) was linearly related to the total increment in accordance with the equation

$$\frac{\text{Increment in old stem}}{\text{Length of the old stem}} = 0{\cdot}0063 \text{ Total increment} + 0{\cdot}039$$

(91 per cent. of the covariance).

6. As a result of this, it is noted that when the trees are small, different correlations of increment with diameter increase may be expected from when they are large.

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Selection for Differing Degrees of Out-breeding in *Nicotiana rustica*

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With three Figures in the Text

ABSTRACT

Initial observations on *Nicotiana rustica* suggested that the percentage of seed set by outcrossing might vary considerably in a segregating population and that this might be due to variation in the position of the stigma relative to the anthers in the corolla-tube. This, measured as the difference in level between the stigma and the ring of anthers, is termed *heterostathmy* and the average expression of this character could be significantly altered by a short programme of selection. These structural modifications were accompanied by developmental modifications affecting the timing of anther dehiscence relative to the opening of the flower. Tests indicated that these changes significantly affected the rate of outbreeding.

INTRODUCTION

THE capacity of a population to respond to the forces of selection depends on its ability to maintain a supply of variability from generation to generation. Such a 'flow of variability', as it has been termed by Darlington and Mather (1949), will be conditioned in higher plants and animals by the average degree of hybridity in the population. This normally will follow from the average relationship of the parents of each individual which in turn will depend on a variety of conditions, the most obvious and most important being the relative frequency of self- and cross-fertilization.

Unless the heterozygous condition has at least twice the selective advantage of the homozygous condition, a system of self-fertilization must finally lead to homozygosity (Hayman and Mather, 1953). The offspring will all be genetically like each other and like their parents and will show high fitness in the environment for which they originally became adapted: but should the environment change the population will not be able to change genetically in the way necessary to give new adjustment. By contrast, cross-fertilization usually results in heterozygosity and hence a flow of variability. Such a population will therefore vary around the optimum genotype and can never achieve the fitness of an inbreeding group; but it will retain a capacity for genetic response to a changing environment and thus a flexibility which is not available to the inbreeder (Darlington and Mather, 1949).

The floral structures of the higher plants are basically designed to enable

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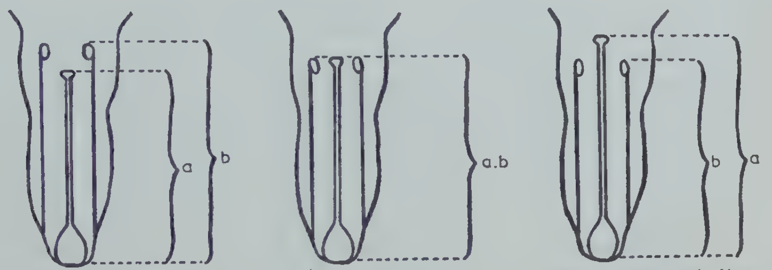
cross-pollination by wind or by insects. During the course of evolution, however, these structures have in many instances become modified in such a way as to affect the breeding system profoundly. Thus, in a number of cases developmental changes have resulted in regular self-fertilization; the failure of flowers to open (cleistogamy), best known in *Viola*, and the opening of flowers only after the pollen has been shed on to the stigma, as in *Pisum* and *Hordeum*, are obvious examples. Regular cross-fertilization, although more difficult to secure, has also been achieved, but the mechanisms have required a greater degree of elaboration than those associated with self-breeding. The most efficient methods depend either on having the sexes on different plants (dioecy) or on some system of self-incompatibility between pollen and style as has been obtained in *Primula*, *Lythrum*, *Trifolium*, &c. Other methods, which discourage rather than prevent self-breeding, are concerned with preventing self-pollination in the place where, with insect-pollination at least, it is most likely to occur—in the same flower (e.g. monoecy, protandry, and protogyny). In these cases self-fertilization can still occur by pollination between different flowers on the same plant.

All these devices, each giving the breeding system which presumably has best met the requirements of the species in its original environment, must obviously have arisen as a result of past selection. By inference it would seem reasonable to suppose that a capacity for such response to selection must still exist, at least in those groups of plants in which the flow of variability has not ceased as a consequence of exclusive self-breeding. On this point, however, no direct evidence has as yet been obtained.

Nicotiana rustica is pollinated naturally by bees and, according to Mather and Vines (1952), about two-thirds of the seed is derived from pollination within the plant, the remaining one-third coming from pollination by other plants. Usually, large quantities of seed from self-pollination can be assured merely by bagging the inflorescence to stop insect visitation. There is, however, considerable variation in the lengths of both style and stamen (see also Paxman, 1956) and this is reflected by variation in the relative position of anther and stigma in the corolla-tube. During inbreeding experiments it was noted that certain plants, characterized by having the stigma set above the anthers in the corolla-tube, failed to set seed in the selfing bags unless artificially manipulated. It was decided, therefore, to see to what extent this feature could be modified by selection and whether, as a consequence, the rate of natural outcrossing would be affected.

METHOD

Selection began in 1952 among individuals of an F_2 generation of *N. rustica* segregating for the duplicate genes which determine the difference between the normal green-leaved plant with a green ovary and the yellowish-leaved plant with a yellow ovary (Mather and Vines, 1952; Vines, unpublished). Selection was confined to the yellows which breed true for this character. Measurements of the difference in level between the stigma and the ring of anthers, termed *heterostathmy* (στάθμη (Gk.) = level; hence, of different levels), were obtained by subtracting stamen length from pistil length, both distances being taken from the base of the superior ovary and using the longest stamen where there were slight differences within flowers. In this way positive values signified a pistil longer than the stamens, thus indicating the superior position of the stigma in the corolla-tube and giving a pin-like appearance; negative values indicated the superior position of the anthers and thus a thrum-like appearance. The use of the expressions *pin* and *thrum* is not intended to convey a closer analogy to the heterostyle condition described in *Primula* and other species than a general morphological similarity. Zero values of this measure signified a condition of homostathmy (i.e. anthers and stigma set at the same level)—see Fig. 1. The basic measurements vary somewhat within plants and Paxman (1956) finds that in this respect the position of the flower on the plant is important. Consequently measurements were always obtained from five flowers taken at random from leading and side shoots, thus ensuring that differences due to position would be taken into account. The mean of these measurements was taken as an indication of the degree of heterostathmy. Although this precaution was thought worth while it must be emphasized that in all cases variation within plants was small compared with that between plants. Selection was practised for both pin- and thrum-like expressions, six lines being initiated by selfing three plants having extreme positively heterostathmic values (*P* lines) and three plants having extreme negative values (*T* lines). Intra-line selection was then continued for a further three generations of self-breeding.



Negative heterostathmy
(a < b)

Homostathmy
(a = b)

Positive heterostathmy
(a > b)

a = pistil length; b = stamen length.

FIG. 1. Diagrammatic representation of heterostathmic and homostathmic expressions.

In 1956 the selection lines (now at F_6) were incorporated in an experimental layout designed to measure the natural rate of outcrossing. The experiment consisted of five replicates each containing a single plant of each of the six lines. The first three replicates were included in turn in the first three blocks of a four-block *N. rustica* stand (designated location *H*). Each of these blocks contained more than 1,000 green plants grown at foot intervals in rows 2 ft. apart and the six yellow plants of the replicate under test were distributed in random order at equidistant points throughout the block and were thus well separated from each other. The remaining two replicates were laid out in a series of isolation plots (location *L*), all plots being well separated from each other and from the main stand. Each isolation plot consisted of five green plants forming a circle 2 ft. in diameter with the yellow plant under test in the centre. The only obvious difference between locations was thus with regard to the numbers of green plants in the vicinity of each yellow plant under test leading, it must be supposed, to a relatively higher availability of foreign pollen in location *H*.

No special provision was made in respect of bee supply but at the time three hives were being maintained on the fringes of the experimental areas and the various replicates were roughly equidistant from one or other of these sources.

Three of the thirty plants put under test failed to reach maturity. Towards the end of the season, ten ripe capsules were taken from each of the remaining twenty-seven plants in such a way as to cover adequately the seed-setting period which extended from mid-July to mid-September. The seed from all ten capsules deriving from a single plant was then thoroughly mixed and a sample of this used to raise a family of a hundred plants in the next season. Twenty-seven such families were thus available in 1957. Since in both locations the yellow (parent) plants were widely dispersed, all but a negligible fraction of any cross-pollination would derive from the contiguous green plants (no colour preference was noted in bee visitations). All the green plants involved were homozygous for the green allelomorph at one or both of the loci controlling the *yellow/green* differences and thus all *green*×*yellow* crosses would yield green offspring; selfing would, of course, give only yellow offspring. The rate of out-breeding of each plant under test could therefore be measured as the proportion of green plants in its 1957 family.

Changes in Floral Morphology following Selection

Heterostathmic measurements were recorded from the parents in 1956 and five of the yellow (and hence selfed) offspring in each 1957 family. The plot mean values of parents (Pa) and offspring (O) are given in Table 1. It should be noted that the parental plot consists of a single plant and the mean is thus based on 5 observations while the offspring plot comprises 5 plants and hence the mean is based on 25 observations. Mean values for the 3 missing plants and their progenies were calculated by minimizing the appropriate *lines*× *replicates S.S.* within locations.

The analyses of variance, based on plot means, are given in Table 2. For each analysis there are in all 29 comparisons between the 30 plot means. Of the 5 comparisons between lines, one is associated with the difference between selection groups (*P* and *T*) and hence measures the overall response to selection; and four are concerned with variation of lines within selection groups, thus measuring the uniformity of this response. One comparison gives the difference between locations and a further five the interactions between locations and the two lines items. Finally, three comparisons can be used to test

TABLE 1

Parental and Offspring Heterostathmic Measurements (pistil length minus stamen length, both in mm.)

Values given are plant averages in the case of parents (Pa) and family averages in the case of offspring (O)

Selection lines		Locations H Replicates A	B	C	L Replicates D	E	Means	
P 1	Pa	2·4	3·3	3·0	2·8	3·4	3·0	
	O	2·3	2·2	2·2	2·1	2·3	2·2	
2	Pa	2·7	1·9	3·3	2·0	2·4	2·5	2·8
	O	2·0	1·8	1·7	1·9	1·9	1·9	2·1
3	Pa	3·3	3·0	3·6	2·7	1·9	2·9	
	O	2·0	2·6	2·5	2·3	2·2	2·3	
T 1	Pa	1·2	0·4	0·9	−0·1	2·3	0·9	
	O	−0·9	−0·8	−0·5	−0·4	−0·2	−0·6	
2	Pa	1·4	0·6*	0·7	0·8	1·7	1·0	0·7
	O	−0·8	−0·9*	−1·0	0·4	0·6	−0·3	−0·2
3	Pa	0·2*	−0·4	0·6	0·1	0·8*	0·3	
	O	0·0*	0·0	0·2	0·3	0·4*	0·2	
Means	Pa	1·9	1·5	2·0	1·4	2·1	1·8	
	O	0·8	0·8	0·9	1·1	1·2	1·0	
	Pa		1·8		1·7			
	O		0·8		1·2			

* Missing plot values—estimated by minimizing the Lines × Replicates S.S. within Locations.

TABLE 2

Heterostathmy—Parental and Offspring Data

Analyses of variance and offspring/parent correlation coefficients—(r)

Item	N	Parental (Pa) M.S.	Offspring (O) M.S.	O/Pa r
Lines (V):				
Selection groups (S)	1	775·21*	1042·65*	0·92*
Lines residual (D)	4	15·85	12·96	
Location (A)	1	0·45	22·84	0·26
V × A:				
S × A	1	23·47	21·47	
D × A	4	5·44	5·27	
Replicates within locations	3	20·55	0·49	
Remainder (basic error)	12	9·31	0·85	

* $P < 0{\cdot}01$ (using D × A M.S. as offspring error).

the consistency of replicates within locations. The remaining fifteen comparisons, involving interaction between lines and replicates within locations, can be taken as a source of error in testing the significance of the other items. This error item has, however, only 12 d.f., 3 having been used in estimating the missing plot values.

The analysis of parental variance is straightforward. The only significant item is that for overall response to selection. There is no evidence that the response differed between lines and nothing to suggest that field layout was anything but random. In the case of the offspring, all M.S.'s in the analysis, except that for replicates within locations, are significantly higher at the 1 per cent. level than the basic error. This analysis, however, must be treated with some caution since the 1957 field layout was not random, the plots being laid out in serial order. Should there be any gradient in field effects certain items, particularly the interactions within locations, would thus very likely be underestimated. Furthermore, while normally the same person was responsible for all measurements in any one year, in 1957 it was necessary to employ a different person in measuring the offspring on plots T1 A–E and T2 A–C. Any personal bias would therefore inflate all items except *replicates within locations* and the *line/replicate interaction within locations*. Consequently, in this analysis, it would be advisable to take the interaction of location and lines within selection groups as the more valid error. Compared with this the only significant item is again that for overall response to selection.

The offspring/parent correlation coefficients are less likely to be affected by the inadequacies of offspring data. These are also given in Table 2. The correlation between lines is significant at the 1 per cent. level and this clearly establishes the heritable nature of the differences between the *P* and *T* selections. In so far as no significant correlation can be detected between plants and their offspring within the lines, it can fairly be assumed that the lines had achieved a considerable measure of homozygosity and were thus relatively true breeding.

The analysis of intra-plot variation is not presented, but in each instance variation within plants is significantly less than variation between plants which itself is of the same order as the between-plot error item.

The analyses leave little room for doubting that certain aspects of floral morphology can be significantly altered by selection. Moreover the character which has been our concern (i.e. heterostathmic expression) must be open to swift and easy adjustment since but four generations of selection superimposed on inbreeding were needed to establish the differences noted, and all three lines at each level responded in a like manner.

It is interesting to note that selection for positive and negative heterostathmic values has not been equally successful (see Table 1). In the *P* lines the stigma is well clear of the anthers, there being an average difference of 2·1 mm. (2·8 mm. for the parents) between pistil and stamen lengths. In the *T* lines, however, an average difference of only −0·2 mm. (0·7 mm. for parents) is obtained, indicating the proximity of stigma and anther in the corolla-tube and thus a homostathmic condition. The significance of this is discussed later.

Changes in Floral Development—A Correlated Response to Selection

During the 1957 season it became apparent that the various families differed in respect of the time of anther dehiscence relative to the opening of the flower. In order to see whether changes in this character were in any way connected with changes in floral morphology it was decided to attempt some measure of its diversity. This was finally achieved by scoring out of the five most recently opened flowers on each plant the number having undehisced anthers. It was appreciated that this measure would be at best but a rough indication of the real situation, but the method had the advantages of simplicity, speed, and less personal bias than most other methods of eye-scoring. The scoring was restricted to families deriving from the *H* location, five plants being scored in each family. The family mean scores are given in Table 3 together with line and selection-group means.

Table 3

*Mean Measurements Relating the Time of Anther Dehiscence to the Opening of the Flower**

Offspring of plants from H location only

Selection lines		Replicates			Means	
		A	B	C		
P	1	0·6	2·6	4·2	2·5	2·7
	2	3·6	2·6	3·2	3·1	
	3	0·8	4·4	2·2	2·5	
T	1	0·2	0·2	0·4	0·3	0·8
	2	0·2	—	0·6	0·4	
	3	—	3·4	0·2	1·8	

* Scored as the proportion of the five most recently opened flowers having undehisced anthers. Five plants counted per replicate.

Since the error variance of a proportion varies with its magnitude the scores have been transformed into angles for the purposes of analysis. The new variables have an error variance depending only on the size of the sample, which in this case is constant, and it is thus possible to calculate a theoretical error variance against which variation from other sources may be tested. The missing plot values have been estimated, after transformation, by minimization of the *lines × replicates S.S.*

The analysis of variance is presented in Table 4. The results must be treated with some caution since, because of the limits imposed on the possible variation by scoring only opened flowers, the data are not normally distributed and cannot be entirely made so by any method of transformation. Because of this the within plot variances tend to be heterogeneous and the average estimate given in the table is thus likely to be spuriously low, although it is still significantly greater than the theoretical sampling variance. The between plot comparisons, however, are likely to be less affected and the interaction M.S. of lines and replicates provides a valid estimate of error. Compared with this the M.S. for selection groups again achieves significance ($P = 0\cdot05\text{–}0\cdot01$), suggesting that selection for heterostathmy has also affected floral development.

The estimates of the correlations between the dehiscence and heterostathmy measurements are more informative. The correlation coefficient between the means of all plants involved (0·69) is significant at the 1 per cent. level. The breakdown (Table 4) shows high and significant positive correlation between lines but none within lines, indicating that during the course of selection a response towards pin-type architecture has been accompanied by increasing retardation in anther dehiscence. From the evidence available it would be difficult to say exactly what underlies this association: because of the low correlation within the lines no physiological concomitancy is implied, but it is impossible to distinguish further between the complex pleiotropic action of a common gene set and the looser association of linked but distinct gene sets. The significance of this association can better be appreciated after consideration of the data on outcrossing.

TABLE 4

Analysis of Variance of Dehiscence Data

(*Transformed to angles and missing plot values estimated by minimizing the $V \times R$ S.S.*)

Item	Variance analysis		Correlation with heterostathmy data	
	N	M.S.	N	r
Lines (V):				
Selection groups	1	23912·10†	4	0·94*
Lines residual	4	815·73		
Replicate (R)	2	3876·43	9	0·24
V × R (error 1)	8	2279·88		
Plants within plots	64	218·09		
Sampling error	∞	164·14		

† $0\cdot01 < P < 0\cdot05$ (using error 1). * $P < 0\cdot01$.

THE EFFECT ON THE BREEDING SYSTEM

The percentage outcrossing (measured as the percentage green plants in the progenies of open-pollinated plants under test) are given in Table 5. For purposes of analysis these data have also been transformed to angles and the missing plot values given in Table 5 have been estimated, after transformation, in the manner already described. It will be observed that the rate of outcrossing ranges from 0 to 70 per cent. with an unweighted mean of 18·3 per cent. (cf. Mather and Vines's estimate of about 33 per cent.).

The analysis of variance takes the form previously outlined and is presented in Table 6. The M.S. for selection-groups is again significantly higher than that for error ($P < 0·01$) and involves a change from an average of 10 per cent. outcrossing for thrum-type selections to an average of 26·6 per cent. outcrossing for pin-type selections. There can be little doubt, therefore, that selection for heterostathmy has brought about changes in the outcrossing potential. The precise relationship between these characters will be further examined below.

TABLE 5

Outcrossing

Percentage hybrid plants from the open pollinated selection lines

		Locations						
		H			L			
		Replicates			Replicates			
Selection lines		A	B	C	D	E	Means	
P	1	12	34	32	13	5	19·2	26·6
	2	17	33	68	9	0	25·4	
	3	30	70	52	14	10	35·2	
T	1	6	5	10	2	4	5·4	10·0
	2	16	25*	12	8	2	12·6	
	3	6*	37	5	9	3*	12·0	
Means		14·5	34·0	29·8	9·2	4·0	18·3	
		26·1			6·6			

* Missing plot values—estimated as before after transforming the data to angles.

TABLE 6

Analysis of Variance of Outcrossing Data

(Percentage hybridity transformed to angles)

Item	N	M.S.	P
Lines (V):			
Selection groups (S) . .	1	1049·03	0·01–0·001
Lines remainder (D) . .	4	110·25	—
Location (A)	1	1731·04	< 0·001
V×A:			
S×A	1	366·37	0·1–0·05
D×A	4	51·67	—
Replicates (R) within locations .	3	231·90	0·1–0·05
Remainder (error) . . .	12	78·78	

The item for locations is also significant at the 1 per cent. level. As previously stated, the only obvious difference between the locations depended on the mass of immediately available foreign pollen. Table 5 indicates that the average percentage outcrossing falls from 26·1 per cent. where the mass is high (*H* location) to 6·6 per cent. where the mass is relatively low (*L* location), suggesting that the percentage outcrossing varies directly with the mass of available foreign pollen. It is interesting to note that Bateman (1947), investigating factors controlling contamination in seed crops, finds the corollary of this to be true: i.e. that the greater the density or mass of intra-varietal pollen the less the contamination by outcrossing with other adjacent varieties.

The item for interaction of selection-groups with locations, although not significant, is suspiciously high and suggests that the lines are disproportionately affected by variation in the mass of available foreign pollen. Table 5 indicates that the higher the outbreeding potential of the lines the greater is the effect of decreased availability of foreign pollen. This in itself is of interest for, although no critical tests were carried out, there were no obvious differences in the total amount of seed set between plants grown in different locations. It would seem, therefore, that a capacity for increased outcrossing in no way diminishes the ability of the plant to set selfed seed under conditions unfavourable to outcrossing. This, of course, we should expect since although the floral structure and development have been altered in certain of the lines in such a way as to discourage self-pollination within the flower, this in no way precludes the possibility of self-pollination from different flowers on the same plant.

One other item, that for variation between replicates within locations, is suggestively high. Since there are no comparable differences with regard to floral structure or development it is not immediately clear why this should be so. The most likely explanation is once more to be found in variation between replicates with regard to the mass of foreign pollen. Certainly, in location *L*, replicate *E* was grown farther away from the main *N. rustica* stand than replicate *D*; while in location *H* the *A* replicate occupied a relatively marginal position in the main stand compared with *B* and *C*.

The relationships between the rate of outcrossing and changes in floral structure and development have been investigated further by means of regression analyses. Since the effects are likely to be more easily recognized when the outcrossing potentials are best realized, attention has been confined to location *H*. The graphs of percentage outcrossing on heterostathmy and on dehiscence measurements (using offspring data, transformed to angles in the case of proportions) are shown in Figs. 2 and 3 respectively. Both regressions are positive and highly significant.

In view of the correlation between the two independent variables a multiple regression analysis has been undertaken and is presented in Table 7. After allowing for variation in the time of anther dehiscence, giving a M.S. which is significant at the 1 per cent. level, no significant residual effect can be traced to heterostathmy. If, on the other hand, we first allow for heterostathmy a M.S. of 1328·80 is obtained, leaving a M.S. of 704·67 for the increment added by simultaneously allowing for anther dehiscence, both of which are significant at the 5 per cent. level. It would appear, therefore, that under the conditions of the test the timing of anther dehiscence is the more important genetic factor in determining the rate of outcrossing. No further residual effects can be traced to differences between lines and thus there is no evidence of other genetic factors affecting this character.

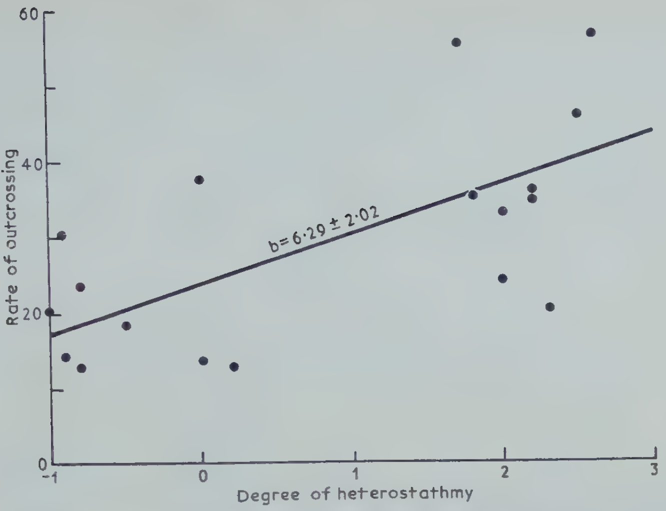


FIG. 2. The regression of rate of outcrossing (angular measurement) on degree of heterostathmy.

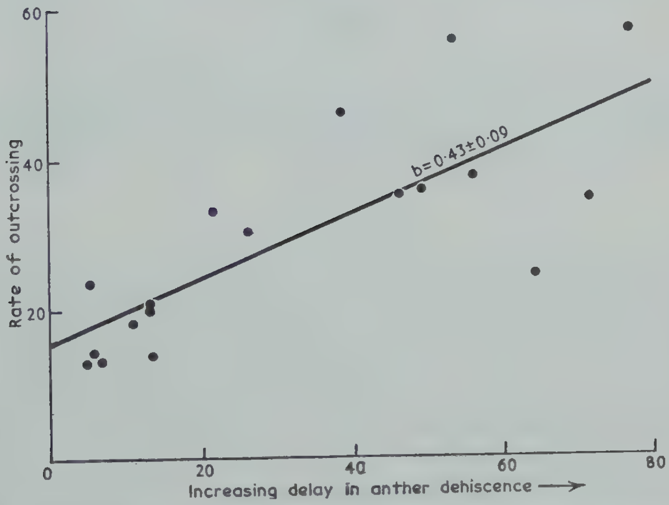


FIG. 3. The relationship between rate of outcrossing and the timing of anther dehiscence relative to the opening of the flower (all data transformed to angles).

As noted, offspring measurements of heterostathmy have been used throughout these analyses and it might well be argued that parental measurements would have been more critical. But, of course, the argument would apply with equal force to the dehiscence data for which no parental measurements were taken. As a matter of interest the data have been re-analysed using parental measurements of heterostathmy (see also Table 7) but the increment ascribable to this character still does not achieve significance.

Since the transformed data have an error variance depending only on the size of the sample (= 100 in all cases) a theoretical error (sampling) variance has been obtained and is appended to Table 7. The residual error M.S. is significantly higher than this, indicating effects other than fluctuations due to sampling. Since Fig. 3 gives no clear indication of anything but a linear relationship between the timing of anther dehiscence and rate of outcrossing, these residual effects must be attributed to the vagaries of bee visitations and to all the other environmental effects noted by Bateman (1947*a*, *b*, and *c*) and Haskell (1944, 1953*a*, 1953*b*, 1954) which this experiment did not seek to measure but included as random variables.

TABLE 7

Control of Outcrossing in N. rustica

Regression and covariance analysis (hybridity and dehiscence data transformed to angles). H location only

Item	N	M.S. using *offspring* data only	M.S. using *parental hererostathmic* data
Timing of anther dehiscence .	1	1964·77*	1964·77*
Increment due to heterostathmy .	1	68·71	223·69
Lines residual	5	77·93	71·10
Remainder (error) . . .	8	104·28	89·17
Theoretical (sampling) error .	∞	8·21	8·21

* $P < 0{\cdot}01$

DISCUSSION

It has been clearly demonstrated that both morphology and development of the *N. rustica* flower can be modified by selection and that as a consequence the rate of outbreeding is affected. On analysis it would appear that change in the relative positions of anthers and stigma is not foremost in affecting the breeding system, the greatest effect being undoubtedly referable to changes in the time of anther dehiscence relative to the opening of the flower. On this point it is of interest to note that rarely does such positioning play a critical part in known breeding devices except in conjunction with some reinforcing system of pollen/style incompatibility, as in the heterostyle *Primula* and *Lythrum* spp. The control of the breeding system by a regulation of the time of anther dehiscence is, on the other hand, comparatively common both as an inbreeding device (e.g. cleistogamy) and as an outbreeding device (e.g. protandry, protogyny).

Nevertheless, even where the system of mating is primarily controlled by a timing device, the positioning of the sex organs must still be important; the proximity of anthers and stigma (homostathmy) being essential for the type of self-breeding instanced above, and a projecting style (positive heterostathmy) obviously facilitating cross-pollination. In this respect two points of significance emerge from the analyses. Firstly, whereas selection for positive heterostathmy was relatively successful, selection for negative heterostathmy did little more than equate the position of anthers and stigma in the corolla-tube resulting in a homostathmic condition. Secondly, positive heterostathmy was associated with retarded anther dehiscence and the homostathmic condition with relatively early dehiscence. It might be expected, therefore, that such a situation would render the breeding system susceptible to swift and easy adjustment and indeed we have shown this to be so. If, as the evidence suggests, the association of the floral characters is based on genetic linkage or pleiotropy rather than on physiological concomitancy, then the situation must have arisen as a result of past selection and must be regarded as an adaptation ensuring rapid response to forces affecting the breeding system. True, the control at all levels lacks the precision of many known self- and cross-breeding devices. But this in itself can be an asset, for the approach to inbreeding still allows a proportion of chance outcrossing and thus maintains the residuum of variability necessary for back selection should conditions change. Also a propensity for high outcrossing does not penalize the plant in terms of seed production when a supply of foreign pollen is not available, for the system does not preclude the possibility of self-fertilization between different flowers on the same plant.

Such a versatile breeding system can to a large extent meet the conflicting demands of immediate fitness and long-term flexibility and thus must confer advantages not given by the more efficient but also more rigid mating systems encountered in other species.

SUMMARY

Under natural conditions *Nicotiana rustica* sets a proportion of its seed by outcrossing, the remainder deriving from pollination within the plant. In segregating populations the position of the stigma relative to that of the anthers in the corolla-tube is not constant and initial observations suggested that the rate of outcrossing might be affected by this variation.

Measurements of the difference in level between the stigma and the ring of anthers (termed heterostathmy) were obtained by subtracting stamen length from pistil length (both distances taken from the base of the superior ovary). In this way a stigma projecting above the anthers was indicated by positive values and the reverse position by negative values. Starting from an F_2 population a simple programme of selection produced lines having a high degree of positive heterostathmy and also some lines which were virtually homostathmic (i.e. both stigma and anthers set at the same level). A correlated response to selection was obtained in so far as anther dehiscence in the homostathmic lines tended to occur at, or immediately preceding, the opening of the flower while in the positively heterostathmic lines anther dehiscence was often considerably retarded.

Tests indicated that the rate of outbreeding under natural conditions was profoundly influenced by the degree of heterostathmy and/or time of anther

dehiscence in that the positively heterostathmic lines with retarded dehiscence had a comparatively high rate of outcrossing. Thus it can be inferred that the rate of outbreeding in this species is under a genetic control which allows swift adjustment to changing conditions. It is suggested that the situation compares favourably with the more specialized but also more rigid mating systems found in other species.

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Photoperiod and Fertility in *Rottboellia exaltata* L.f.

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With two Plates

ABSTRACT

In the short-day grass *Rottboellia exaltata* L.f., one of the flowers of the sessile spikelet at each node of the inflorescence is morphologically male and the other hermaphrodite. When plants are exposed to 12 weeks of long days before induction to flower in short days, the male flower is sterile, and self-pollination occurs in the hermaphrodite flower before exsertion of the anthers. With 17 weeks of long days before exposure to short days, the male flower becomes fertile in the first-formed inflorescences, and some of the hermaphrodite flowers are probably open to cross-pollination.

INTRODUCTION

IN many polygamous grasses the distribution of the sexes in the inflorescence is inconstant, varying in the same individual during the course of the flowering season. This variability is likely to have significant effects on the breeding system, and it is of some interest from the points of view both of developmental physiology and population genetics to discover to what extent it is governed by environment. Through the kind co-operation of Mr. C. E. Hubbard of the Royal Botanic Gardens, Kew, several short-lived polygamous grasses have been assembled, and the effects of various combinations of environmental conditions upon their sex expression are being tested. The present paper concerns the effects of photoperiod on *Rottboellia exaltata* L.f., of the tribe Andropogoneae.

MATERIALS AND METHODS

The strain of *R. exaltata* used was of Australian origin, C.S.I.R.O. Lot No. 813 C959. Caryopses were germinated in a sand-peat mixture, and the seedlings transferred at an age of 5 or 6 days to individual plastic containers where they received 65 ml. of a balanced nutrient solution containing *c.* 100 p.p.m. nitrogen every 3rd day, with morning watering on the intervening days. From 9 a.m. to 5 p.m. daily, the plants were in natural daylight, with air temperatures varying between 22° C. and 35° C. and relative humidity maintained above 60 per cent. Between 5 p.m. and 9 a.m., plants receiving short days were transferred to air-conditioned dark chambers at 22° C. and 70 per cent. relative humidity. Those under long days were maintained in the

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same air temperature and humidity, but received natural daylight supplemented with light at *c.* 100 f.c. from incandescent sources to give a total day length of *c.* 20 hours.

All plants were grown to an age of 12 weeks in long days, by when they were 30–35 cm. in height, with the 4th leaf just emerging. A group of twelve (Series A) was transferred to short days at this time, and a further group of twelve (Series B), 5 weeks later. A third group of twelve plants remained on long days throughout the 30-week period of the experiment.

OBSERVATIONS

Flowering behaviour. Flowering began in the plants of Series A (short days from an age of 12 weeks) after 34–38 short days, when the mean number of nodes on the principal axis was $9{\cdot}33 \pm 0{\cdot}140$ and in Series B (short days from an age of 17 weeks) after 28–32 short days, the mean node number of the principal axis then being $12{\cdot}50 \pm 0{\cdot}144$. The plants maintained on long days throughout remained strictly vegetative, and dissection at an age of 30 weeks revealed no inflorescence primordia. So far as the initiation of flowering is concerned, this strain of *R. exaltata* has thus a photoperiodic behaviour of a normal short-day type.

Flower structure. The inflorescence is of a pattern characteristic of many Andropogoneae, spike-like and segmented, each segment bearing two spikelets, one sessile and the other stalked (Plate 1A). In *Rottboellia*, the pedicel of the stalked spikelet is fused throughout its length to the rachis; the spikelet itself is two-flowered, male or sterile. The sessile spikelet is two-flowered, both flowers lying in a notch in the rachis protected by the outer glume and the pedicel of the stalked spikelet. The inner flower (morphologically the lower) is characteristically hermaphrodite, the outer male or sterile.

In no instance in this experiment was fertile pollen formed in the morphologically male flowers of the stalked spikelets, although there was some variation in the development of the anther rudiments. In the sessile spikelets, the inner hermaphrodite flowers remained consistently fertile in both sexes in all plants of the two flowering series. The outer male flowers, however, differed in fertility under the two photoperiodic treatments. Their average state at the time of exsertion of the anthers of the hermaphrodite flowers was recorded for every plant in the first emerging inflorescences of the principal axis. The outer glumes and lemmas were folded back to expose several flowers in the central region of the inflorescence, and the development of the anthers scored according to a four-point scale as follows: (1) anther rudiments less than 0·75 mm. and obscured by the lodicules; (2) anther rudiments 0·75–1·25 mm., visible behind the lodicules; (3) anthers 1·25–1·75 mm.; (4) anthers exceeding 1·75 mm. In any one inflorescence the state of the male flowers as assessed by this scoring method was usually quite consistent, and the distributions of the plants of the two series for the first inflorescences at the second and third flowering nodes of the principal axis are given in Table 1. At both nodes at the time of anthesis of the hermaphrodite flowers the anthers of the male flowers were considerably further developed in the plants receiving the greater number of long days before induction to flower in short days.

Pollen fertility. Pollen formation was followed with the use of acetic-orcein squashes and sectioned material stained in haematoxylin. Meiosis was normal in all of the anthers examined of the hermaphrodite flowers of the sessile spikelet. Characteristically, as the pollen ripens the anthers assume an orange-red colour, and at this time the grains become packed with starch.

In the anthers of the outer flowers of the sessile spikelets, the state of the pollen could be correlated reasonably well with anther development as recorded in the grades of Table 1. Anthers which had not developed beyond grade 2 were generally found to have been arrested in pre-meiotic or early meiotic stages; the contents usually formed an irregular plasmodium surrounded by degenerating tapetum. In anthers corresponding to grade 3 pollen grains were present, but commonly highly vacuolated and without starch. Some anthers contained grains of varying size (Plate 1C), and in others the pollen had collapsed. The pollen of anthers exceeding 1·75 mm. in length (grade 4) was morphologically normal. This grade represents merely a late phase of pollen development before the accumulation of starch in the grains, and such anthers later ripen normal pollen (Plate 1B).

TABLE 1

Anther Sizes in Male Flowers of the Sessile Spikelets of Inflorescences at the Second and Third Flowering Nodes of Rottboellia exaltata *Flowering in Short Days after (A) 12 weeks of Long Days, and (B) 17 weeks of Long Days. Twelve plants per series*

Series	Flowering node	Anther size grades (1) < 0·75 mm.	(2) 0·75–1·25 mm.	(3) 1·25–1·75 mm.	(4) > 1·75 mm.
A	2nd	12	0	0	0
	3rd	12	0	0	0
B	2nd	0	5	4	3
	3rd	1	2	5	4

Periodicity. In the hermaphrodite flowers of Series A, the anthers regularly dehisced before anthesis. A proportion of the pollen germinated on the stigmata before their emergence, so it may be assumed that with this photoperiodic experience, *R. exaltata* is commonly self-pollinating. The anthers were later exserted, followed in the course of an hour or two by the stigmata. An unusual feature is that the stigmata and anthers are withdrawn between lemma and pale after flowering, where they remain during the growth of the caryopsis to become crushed eventually against the roof of the notch in the rachis. In plants of Series B, whilst the possibility of a high proportion of internal pollination before anthesis could not be excluded, in at least some of the hermaphrodite flowers the anthers dehisced *after* exsertion. The anthers of the outer flower were exserted and dispersed pollen 4–8 days after the withdrawal of those of the inner flower, at a time when the caryopsis was already well grown. The anthers of the outer flower were not subsequently withdrawn, but remained exserted until the disarticulation of the rachis.

Fertility of later inflorescences. During the period of flowering, inflorescences are produced in succession on a series of short lateral axes arising from the upper nodes of the main stems. The observations of Table 1 refer to the first inflorescences of the main axis to emerge after transfer to short days, but for a number of selected plants the fertility of male flowers in later inflorescences was also examined. The plants of Series A showed no variation throughout the flowering period, the male flowers remaining consistently sterile. In the three of those of Series B examined, fertility in the male flowers of later inflorescences produced in short days fell progressively, and in all cases those of the fourth-order inflorescences of the main axis were totally sterile.

DISCUSSION

These results indicate that in the short-day grass *Rottboellia exaltata* sex expression, and probably also the breeding system, can be modified through the agency of photoperiod. With 12 weeks of long days before induction to flower in short days, pollen fertility is restricted to the anthers of hermaphrodite flowers and self-pollination takes place internally before anthesis, but with 17 weeks of long days before transfer to short days the male flowers of the sessile spikelets also become fertile to varying degrees, and some at least of the hermaphrodite flowers are probably open to cross-pollination. The trend in sex expression, in so far as it represents a depression of maleness in conditions promoting early flowering, is comparable with that known in other short-day plants (Heslop-Harrison, 1957). It can be related also to the reactions of the long-day species, *Silene pendula*, in which full male fertility is obtained only when plants are exposed to 40–50 short days in early life before induction to flower in long days (J. and Y. Heslop-Harrison, 1958*a*, 1958*b*).

In *Silene pendula*, a native of the Mediterranean regions, this behaviour is likely to have an effect upon the breeding system in natural populations, since in the wild plants arising from autumn and early spring germination will be hermaphrodite, and those germinating after the equinox are likely to be male sterile in varying degree. The implications in *Rottboellia* so far as the breeding behaviour in natural populations is concerned are not so clear. *R. exaltata* is a plant of the Old World tropics, and it is probable that the amplitude of day-length variation which it experiences in natural habitats is small compared with that in the present experiment. Further experiments are planned with *Rottboellia* and other polygamous tropical grasses to explore the effects of day-lengths varying within smaller limits.

ACKNOWLEDGEMENTS

It is a pleasure to thank Mr. C. E. Hubbard, who suggested *Rottboellia* as material suitable for these experiments, for supplying the material and for taxonomic advice. My thanks are due also to Miss I. Woods, B.Sc., for her efficient technical assistance, and to Mr. Robert Johnston for tending the plants.

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EXPLANATION OF PLATES

PLATE 1

A. Single segment of the spike-like inflorescence of *Rottboellia exaltata.* A, stalked spikelet; B, sessile spikelet; C, rachis; D, pedicel of stalked spikelet fused to rachis. (× 12)

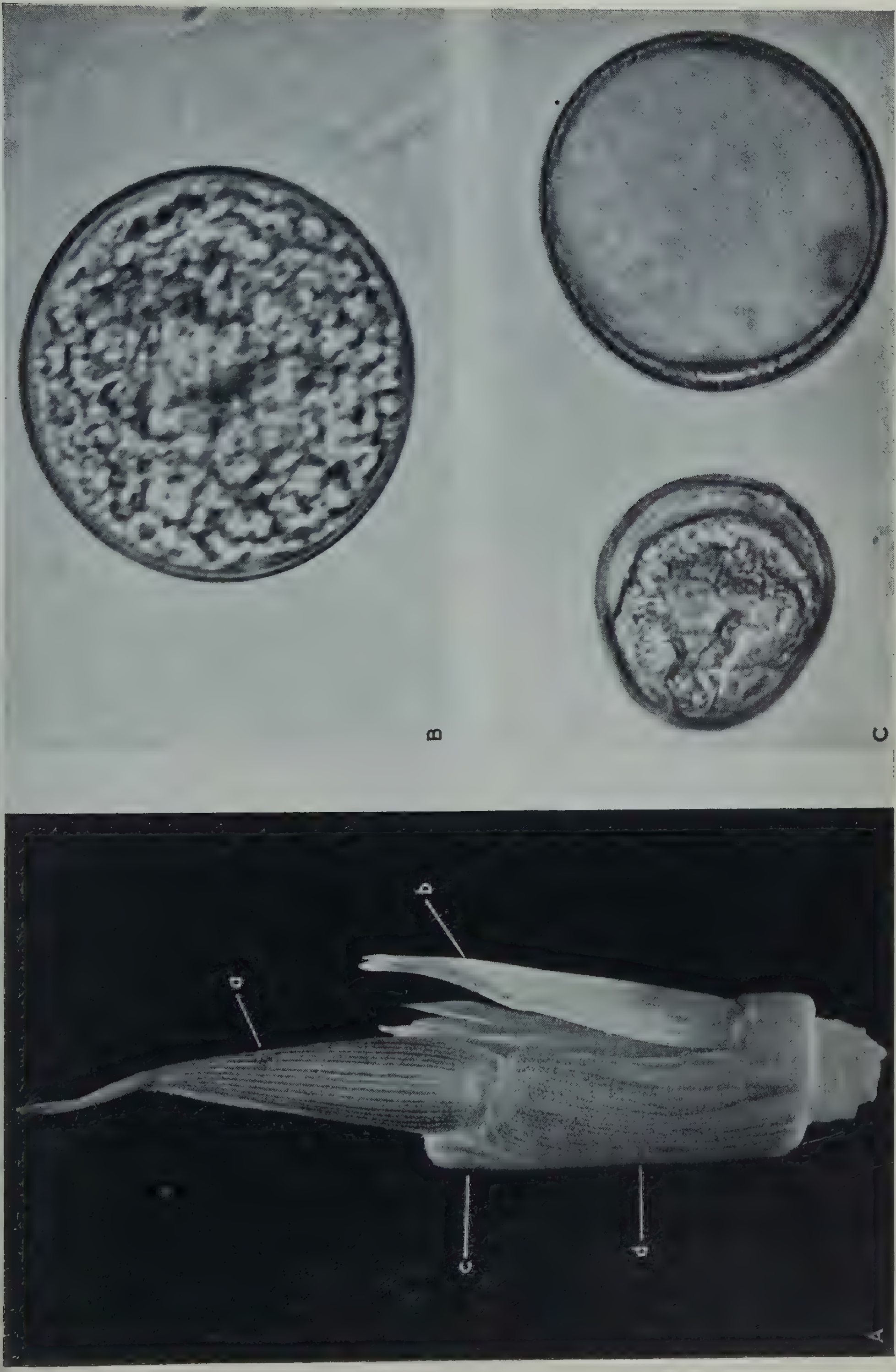
B. Normal starch-filled pollen grain from the outer flower in a sessile spikelet of a plant kept in long days for 17 weeks before induction to flower in short days. (× *c.* 1,200)

C. Abnormal pollen grains from an imperfectly fertile male flower of a sessile spikelet. (× *c.* 1,200)

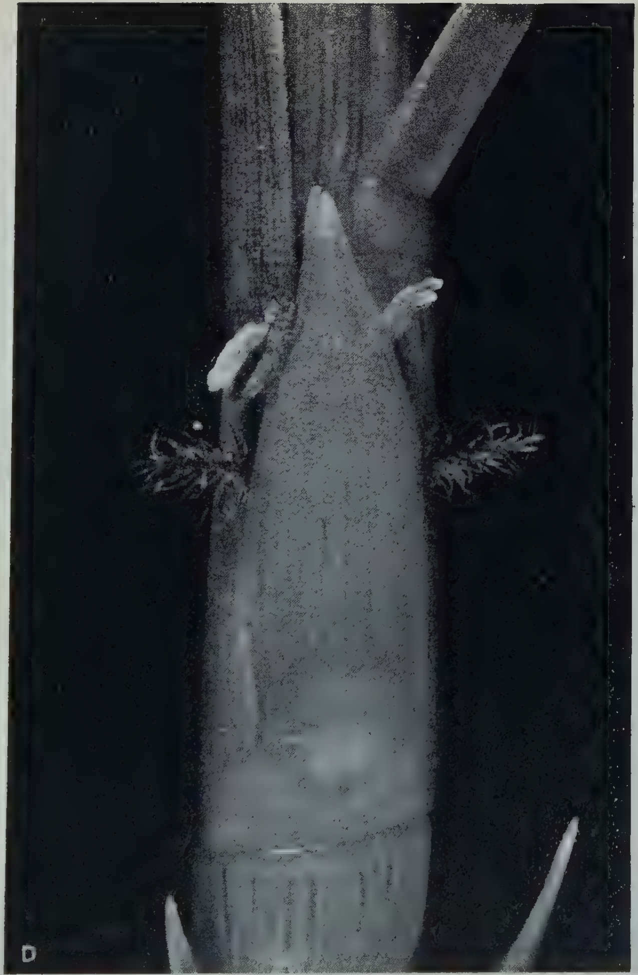
PLATE 2

D. Anthesis of the inner hermaphrodite flower of a sessile spikelet in a plant given 12 weeks of long days before induction to flower. The anthers have dehisced internally before exsertion, and the outer flower is totally sterile. (× *c.* 15)

E. Anthesis of the fertile outer flower of a sessile spikelet in a plant given 17 weeks of long days before induction to flower. The stigmata and anthers of the inner hermaphrodite flower have already been withdrawn. (× *c.* 15)



J. HESLOP-HARRISON



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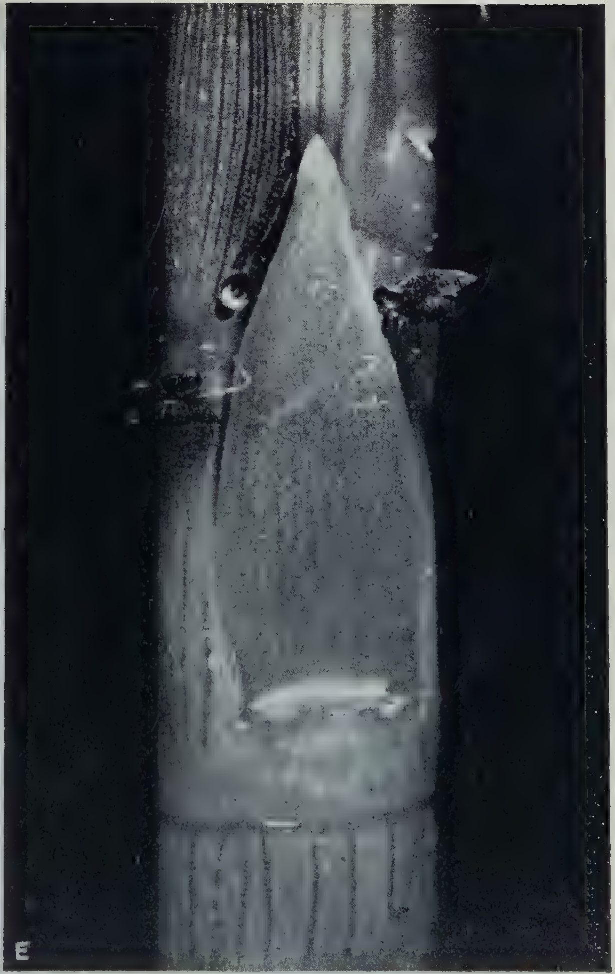

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J. HESLOP-HARRISON

Evidence for a Flower Inhibitor and Vegetative Growth Promoter in the Strawberry

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With one Figure in the Text

ABSTRACT

Experiments with pairs of strawberry runner plants joined by the stolon and acting as donor/receptor units are reported. Long photoperiods or a light-break treatment on donor plants brought about increased petiole length, increased leaf size, and delayed flower initiation in receptor plants themselves in short photoperiods. The response of receptor plants was increased by exposing donor plants to illumination by full daylight 3 hours earlier each morning than receptor plants. It is thought that this earlier illumination increased translocation from the earlier to the later illuminated plants. In translocation experiments, radioactive P^{32} moved more freely from the older to the younger plant of each pair, except when the older plants were partially defoliated or when the younger plants were exposed to full light for a longer period of time each day than the older attached plants.

The results are discussed and it is concluded that they are good evidence for the existence of a growth-regulating substance(s) which promotes vegetative growth and inhibits flower initiation. It seems possible that the regulation of vegetative growth and the control of flower induction is principally achieved in the strawberry by a flower inhibiting, vegetative growth-promoting system.

INTRODUCTION

FLOWER induction in plants sensitive to photoperiod is thought to result from the action at the growing apices of specific flower-promoting substances or hormones. The existence of these substances is inferred from the fact that photoperiodic perception is located in leaves, whereas the response occurs at the growing apices, thus implying a translocatable chemical stimulus or hormone.

Evidence is now offered for the existence in strawberry plants of translocatable substance(s) whose activity in the growing regions inhibits flower formation and at the same time promotes increases in leaf size, petiole length, and stolon production. In the original experiment in this investigation (Guttridge, 1956) the characteristic features of growth in long photoperiods, namely, long petioles, stolon production, and inhibition of flower formation, were induced in plants exposed to short photoperiods (receptor plants) by a continuous-light treatment given to daughter plants (donors) attached to them by the natural stolons. Since the promotion of vegetative growth in the receptor plants in short photoperiods was proportional to the number of

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leaves retained on the donor plants in continuous light, it was impossible to separate photoperiodically induced effects from nutritional effects caused by greater photosynthesis in the plants with greater leaf areas. However, the appearance of the plants suggested a formative rather than a nutritive effect.

Since then, four further experiments have been carried out, the results of which support the main observation that plants in long photoperiods can stimulate vegetative growth and delay flower initiation in plants attached to them by stolons. One of these experiments only is reported in detail as it and the original one together illustrate the principal features of the investigation. In another of these experiments illumination of donor plants by full daylight 2 hours earlier each day than receptor plants increased the response of receptors, possibly by increasing translocation of the vegetative stimulus by promoting movement of assimilates from donor to receptors. This experimental technique was incorporated in the present photoperiodic experiment. Since translocation is clearly an important factor in experiments with donor/receptor units, attempts were made to determine the movement of P^{32} in donor/receptor units similar to those used in the photoperiodic work. The results support deductions made from the photoperiodic data concerning the direction of translocation between members of a pair under various environments.

MATERIALS AND METHODS

Two varieties, Royal Sovereign and Redgauntlet, of the cultivated strawberry (*Fragaria ananassa* Duch.) were used in various experiments. Plant pairs were obtained by rooting adjacent runner plants from a runner series in separate clay pots plunged in the field. The selection of adjacent runner plants, discarding the original mother plant, gave units in which the difference in size was minimal. After rooting the pairs were severed from the original mother plant. The stolon joining the members of the pair was carefully preserved intact. When the older plant of each pair described here as the 'parent' plant had 2–3 expanded leaves, the pairs were ready for treatment. The younger daughter plants had 1–2 leaves at this time.

The experiments were carried out in a heated glasshouse. Low-sided boxes each holding five pairs of plants were fitted with wood and wire frames over the plants. The frames supported light-proof black cloth which was put in place each evening and removed each morning according to the light régime required. The frames were arranged so that different light treatments could be given to donors and receptors. One Tungsten filament lamp was fitted under the superstructure of each box for light-break treatments during otherwise long dark periods. Dark controls received an equivalent amount of overhead heat from lamps of the same wattage enclosed in light-proof canisters.

The results were analysed separately for parent, daughter, donor, and receptor plants, as the variances of these groups were thought to differ significantly. In fact, the variance differences were not as large as expected, but as clear-cut results were obtained using the separate variances there seemed little point in pooling merely to increase the available degree of freedom.

In the experiments with radioactive phosphorus the pairs of plants were placed in the appropriate environments in growth cabinets at least 1 week before treatment. P^{32} was applied by brush as orthophosphoric acid or as the potassium di-hydrogen salt in aqueous solutions together with a spreader to the upper and lower surfaces of selected leaves of one plant of each pair. Different amounts of P^{32} were applied in different experiments, the maximum being about 9 μc. per plant. The laminae of emerging leaves on receptor plants were harvested after appropriate periods of time, dried, and autoradiographed. The tri-laminate structure of the leaves allowed three harvests at intervals, giving a rough assessment of the accumulation of radioactivity.

EXPERIMENTAL WORK

Photoperiodic experiment

Plants of the variety Redgauntlet were rooted in 5-in. pots in the field early in August and brought into the glasshouse on August 21, when the experiment was started. Five pairs of plants were accommodated in each box, three arranged with daughter plants as 'donors' and two the reverse way. Each box was considered as a plot for statistical analysis. With three replications there were fifteen pairs of plants in each treatment.

During treatment the plants received good glasshouse culture and interference was restricted to the cutting back of stolons and removal of the occasional developing branch crown.

On November 7 the plants were harvested and dissected, each having produced 6–8 leaves during treatment. Records were made of petiole length, measured from the insertion of stipules to the junction of lateral leaflets; length and breadth of each leaflet; stolon formation and the number of leaves formed from the start of treatment to flower initiation. (Inflorescences being formed terminally in the strawberry.)

There were two treatment variables. One was photoperiodic and consisted of a light break of 3 hours' duration in the middle of each dark period at an intensity of 25–45 f.c., with control plots in a long uninterrupted dark period. The other was designed to increase translocation from one member of a pair to the other. The black cloths were removed 3 hours earlier from one member of a pair than from the other, thus allowing an earlier build up of photosynthates and creating a concentration gradient from the earlier to the later illuminated plants. From the results it appears that translocation from the earlier to the later illuminated plant was in fact increased. Both the shorter 7-hour and the longer 10-hour photoperiod used are photoperiodically short to the strawberry.

Four treatments were arranged to give three independent comparisons, each between two treatments (Table 1).

Treatments A and B differ only in the photoperiodic treatment of 1 plant of each pair. Treatments B and C differ only in the duration of daylight received by 1 plant of each pair. In B the plants receiving a light break also have the advantage of earlier photosynthesis. Treatments C and D differ in duration of daylight, but in D the plants in short photoperiods have the advantage of earlier photosynthesis.

Minimum night temperatures in the illuminated sides of boxes averaged 1° C. higher in treatments B and D and 0·6° C. higher in treatment C (lamp in light-proof canister) than the temperature in the long-night sides of the boxes. The daily minimum temperatures were between 10° C. and 15° C. except occasionally. Spot thermograph checks generally showed a steady fall in temperature under the cloths from sunset until soon after sunrise, with a check in the fall, or even a slight rise during the light break. During the early part of the experiment the temperatures rose sharply in bright sunshine during the short period from the time the cloths were put in place until sunset.

TABLE 1

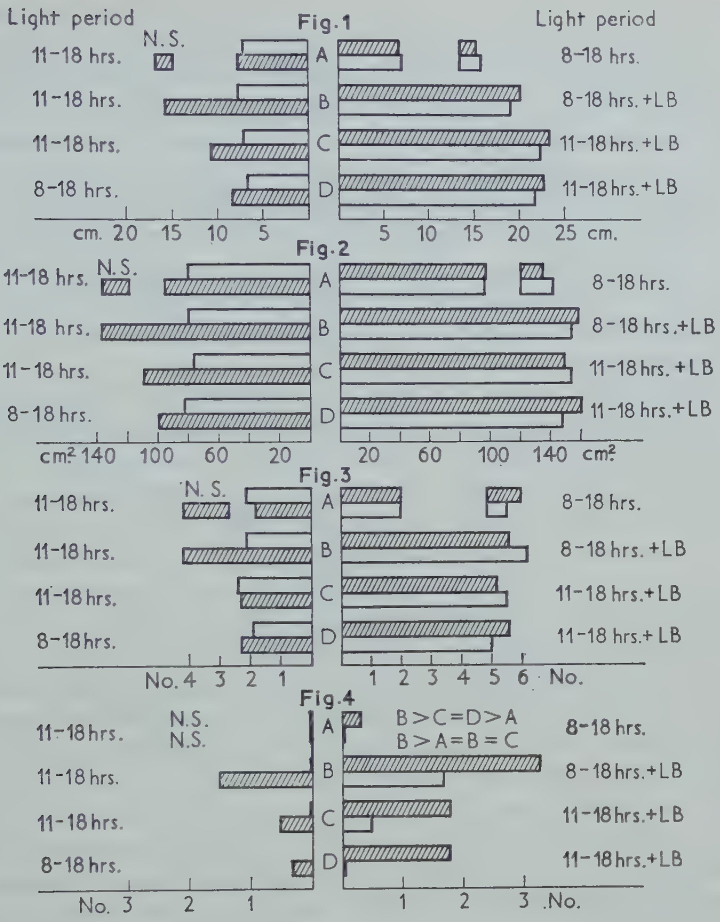
Table of Treatments

	One member of pair		Other member of pair	
Treatment designation	Duration of daylight	Photoperiod	Duration of daylight	Photoperiod
A	7 hours	short	10 hours	short
B	7 ,,	,,	10 ,,	,, +light break
C	7 ,,	,,	7 ,,	,, + ,,
D	10 ,,	,,	7 ,,	,, + ,,

All results are presented diagramatically. The effect of direct exposure to a light break is shown in the comparisons between treatments A and B on the right-hand side of each figure. Petiole length (Fig. 1) was more than doubled, leaf size was increased by about half (Fig. 2), flower initiation was delayed by about four leaves (Fig. 3), and stolon production increased (Fig. 4).

The remote effect of a light break on attached plants is shown on the left side of each figure, again comparing treatments A and B. Petioles were about doubled in length in daughter plants by the light-break treatment of parent plants attached to them, but there was no effect on parent plants when their daughter plants received a light break. Apparently parent plants acted as effective donors; daughter plants did not. Leaf size, number of leaves to the first flower truss, and stolon production were also increased in daughter plants by the light-break treatment of parent plants, although the difference in stolon production was not significant.

Comparing treatments B and C it is clear that the response of 'receptors' (left side of the figures) to a light-break treatment of 'donors' (right side) is increased by exposing 'donor' plants each day to full daylight 3 hours earlier. The differences are significant for petioles, leaf size, and number of leaves to the first flower truss, but they are again restricted to arrangements with parent plants receiving a light break, that is acting as 'donors'. It is thought that the



FIGS. 1–4. Parent plants (clear histograms) are represented opposite the daughter plants attached to them (shaded histograms). Histograms of parent plants on the left side represent means of 9 plants, and on the right side, 6 plants: the numbers of daughter plants are the reverse. Significant difference ($P = 0{\cdot}05$) are indicated separately for parent and daughter plants, against the A treatment histograms. NS indicates differences are not significant. The lighting treatments (actual times of exposure to daylight) are shown adjacent to the respective histograms LB = light break.

FIG. 1. Petiole lengths. Means of third to sixth leaves emerging on each plant during treatment.

FIG. 2. Leaf areas calculated from $\frac{1}{4}(\text{Length}+\text{Breadth})^2\pi$. Means of third to fifth leaves emerging on each plant during treatment.

FIG. 3. Mean number of leaves per plant initiated before the first inflorescences (5 leaves assumed in the bud).

FIG. 4. Mean number of stolons per plant emerging during treatment.

photosynthetic advantage of earlier daylight exposure assisted translocation of the vegetative stimulus.

The plants in short photoperiods in treatment D were given 3 hours longer daylight each morning than plants under light-break treatment attached to them. This was expected to give a photosynthetic advantage to the plants in short photoperiods, encouraging the translocation of a flower-inducing and vegetative growth-restraining substance to plants under a light-break treatment. Comparison with treatment C shows that the improved translocation in treatment D did not affect the behaviour of the plants receiving a light-break treatment (right side of figures). The translocation of a flower-inducing substance along the stolon was not therefore detected in this experiment. It is possible that the environmental conditions were too extreme for a flowering response, although this should not have precluded effects on petiole length and leaf size.

As already noted, stolon production was promoted by the light-break treatment of plants directly illuminated, although individual records show that not all plants produced stolons. Also, in both parent and daughter plants directly illuminated by the light break, stolon production was greater in treatment B than in treatment C (right side of Fig. 4), presumably due to the nutritional benefit of 3 hours extra daylight each day. The individual plant records show that both initiation and emergence of stolons was affected. The promotion of stolon production in 'receptor' plants in treatment B (left side of Fig. 4) just failed to reach significance. The small response may have been due to too low temperatures and also to the nutritional effect of the short 7-hour day. Darrow (1936) showed that stolon production is favoured by high temperatures, and this has since been confirmed (Smeets and Kronenberg, 1955; Went, 1957).

Earlier experiments gave significant increases in petiole length due to long-day treatment of attached plants, although delays in flower initiation and increases in stolon production were not significant. The limited success of these earlier experiments is probably due to environmental conditions, especially temperature, being insufficiently favourable for vegetative growth, so that the threshold levels of stimulation necessary for stolon production and inhibition of flowering were not reached. Inhibition of flowering and promotion of stolon production were significant in the previously reported experiment (Guttridge, 1956). Leaf size was not recorded in these earlier experiments.

Experiments with P^{32}

The first experiment was intended to indicate suitable rates and times of application of P^{32} and suitable harvest times. The result, however, also showed clearly that P^{32} moved more freely from parent to daughter plant than in the reverse direction when both were in the same environment. Activity was detected in daughter plants 24 hours after application of P^{32} to parent plants. No activity was detected at equivalent film exposures in parent plants 7 days after application to attached daughter plants.

In the second experiment illumination was arranged so that 'donor' plants received light 3 hours in advance of receptor plants each day. Both received a 15-hour photoperiod. Five pairs were arranged with parents and five with daughters as 'donors'. A single application of P^{32} was made to 'donors' near the end of their light period. No activity was detected in receptors 15 hours later. Slight activity appeared 19 hours after application and considerably more 24 hours after application but only in units where parent plants were treated with P^{32}. In contrast the first signs of activity in parent plants following treatment of daughter plants appeared after 4 days, none being detected in leaves harvested after 15, 19, 24, or 48 hours.

The third experiment was arranged to study the movement of P^{32} from daughter to parent plants when (*a*) daughter plants were in longer light periods (17 hours full light) than the parent plants (9 hours full light) and (*b*) when parent and daughter plants had very different leaf areas, achieved by removing all leaves older than the most recent fully expanded leaf from one plant. Intact plants had 4–5 fully expanded large leaves. Including controls, six treatments were set up, with 3–5 plants in each treatment. Leaves were harvested 31 hours and 55 hours after application of P^{32}, which was to the youngest fully expanded leaf on each 'donor' plant. The results show that under the conditions of the experiment, (1) P^{32} moved from intact parent to intact daughter plants but not in the reverse direction when both were in the same 9-hour day; (2) P^{32} moved strongly from intact daughter plants to defoliated parent plants but not from defoliated daughter plants to intact parents when both were in a 9-hour day; (3) P^{32} moved from intact daughter plants in a 17-hour day of full light to intact parent plants in a 9-hour day. Movement under the same circumstances was detected in one pair of plants when the daughter plant was defoliated. In summary it appears that P^{32} moved freely from parent to daughter plant but not in the reverse direction unless (*a*) the parent plant was defoliated, (*b*) the daughter plant was in a 17-hour and the parent in a 9-hour light period.

Clearly in these experiments radioactive phosphorus moved more readily from parent to daughter than in the reverse direction. There is also a good correlation between the movement of phosphorus and the expected movement of assimilates under the various experimental circumstances.

DISCUSSION

In this discussion the promotion of petiole length, leaf size, and runner production, and the delay in flower initiation will be referred to collectively as the promotion of vegetative growth.

It is clear from the results that a light-break treatment promotes vegetative growth not only in plants directly exposed to it, but also in receptor plants attached by stolons. Increased promotion of vegetative growth is observed if the plants receiving the light break are exposed to daylight 3 hours earlier than their attached receptor plants. It is very probable that this increased response results from increased translocation of the stimulus. Earlier illumination without a light break was ineffective.

Two basic interpretations of the observed facts are possible. The vegetative growth of plants in short photoperiods was increased either (1) by promotive substances received via the stolon from plants receiving a light break, or (2) by the draining away from plants in short photoperiods of substances which inhibit vegetative growth (although possibly promoting flower initiation). The former interpretation involves translocation from the plants receiving a light break to the plants in short photoperiods. The alternative interpretation involves movement in the opposite direction.

The first interpretation seems the more probable for two reasons. (1) It is likely that the older parent rather than the younger daughter plant of a pair will act as donor, this being the natural relationship in vegetative propagation by stolons. If this supposition is accepted then the greater treatment effect would be expected in parent plants if the daughters were receiving inhibitors, but in the daughter plants themselves if a promotive substance was involved. In the experiment the response was the greater when the daughter plants themselves were stimulated into increased vegetative growth, thus suggesting that they were receiving a growth stimulant. (2) It seems more probable that the donating rather than the receiving capacity of plants would be enhanced by 3 hours earlier full daylight each morning, although this would only apply if the stimulus moves with the assimilate stream as is suggested.

This suggestion is supported by the fact that a considerable range of both naturally occurring and synthetic substances has been shown to move through plants under circumstances and in directions in which photosynthates might be expected to move. These substances include curly top and other viruses (Bennett, 1937); 2,4D (Mitchell and Brown, 1946; Crafts, 1956; Barrier and Loomis, 1957); Schradan (Thomas and Bennett, 1954); reproductive stimuli (Stout, 1945; Carr, 1957); P^{32}, probably as organic compounds (Norton, 1954; Barrier and Loomis, 1957).

The experiments reported also suggest that radioactive phosphorus moved with the assimilate stream in the strawberry and certainly indicate freer movement from parent to daughter plant than in the reverse direction. Thus there are substantial grounds for accepting the hypothesis that vegetative growth was increased in receptor plants by the activity of a promotive substance.

In the earlier experiment (Guttridge, 1956) vegetative growth was promoted in intact parent plants following long-day treatment of daughter plants, some of which were partially defoliated. These circumstances would appear to favour translocation from plants in short days to the plants in long, thus suggesting the draining out of inhibitors. It is probable, however, and the third experiment with P^{32} is cited in support, that the excess duration of daylight received by the plants in long days (natural day supplemented to 24 hours with incandescent light), compared with the plants receiving a 9-hour day only, was sufficient to ensure translocation of assimilates at least during part of each day towards the responding plants.

The experimental facts together with these considerations suggest that vegetative growth in the strawberry is stimulated by the activity of a vegetative growth-promoting substance(s), which is formed in long photoperiods and can be translocated along the stolon. Flower initiation is delayed while runner production, petiole length, and leaf size are increased by the activity of this substance.

The inclusion of stolon production among the features of growth promoted by this hypothetical substance would hardly be justified by the results of the experiments reported here were it not for the fact that the promotion of stolon production by direct exposure to long photoperiods is well established. It seems unlikely that the other characteristic feature of growth in long photoperiods—long petioles, large leaves, and delayed flower induction—are affected by a substance produced in long photoperiods while stolon production alone is not actively promoted by long photoperiods but only inhibited by short photoperiods.

The failure to detect the activity of a supposed flower-*promoting* substance in the photoperiodic experiment and the failure to induce flower initiation in the earlier reported experiment (Guttridge, 1956) except under the extreme circumstances of almost complete defoliation of receptor plants suggests that the vegetative growth-promoting and flower-inhibiting system may be the dominating influence in the photoperiodic responses of the strawberry varieties Royal Sovereign and Redgauntlet. Hartmann (1947) produced evidence also from an experiment with pairs of plants, apparently suggesting the translocation of a flower-promoting substance along the runner. In the variety Missionary, flower initiation was induced in young runner plants in long photoperiods when their mother plants were exposed to short photoperiods. As defoliation of the runner plants had no effect, however, it is possible that induction occurred at an early stage before the runner plants had developed leaves large enough to respond to the long photoperiod. Under these circumstances a flower-inhibitory system could not be expected to operate and induction of flowers could have occurred in the absence of inhibitors.

It is therefore possible that the regulation of vegetative growth and the control of flower induction in the strawberry is principally achieved by a flower-inhibiting, vegetative growth-promoting system and that flower induction is genetically induced in the absence of sufficient inhibitor. This hypothesis is not incompatible with Went's data (1957) on the effect of temperature on flower induction in strawberry. Detailed discussion of the temperature effects will be postponed until more recent experiments are reported.

Comparable evidence for positive stimulators of vegetative growth and inhibitors of flower induction has been obtained with *Kalanchoe*. Schwabe (1956) has shown in *K. blossfeldiana*, a short-day plant, that a single long-day interrupting a series of short-day cycles has a positive inhibitory effect on the flowering response and is not just neutral. This work follows extensive investigations by Harder which provided evidence for a chemical process at work in the plants which was inhibitory to flowering, and also of a second process inducing the vegetative responses associated in this species with long photoperiods. Both *K. blossfeldiana* and the cultivated strawberry plant alike have strongly developed vegetative responses to photoperiod. Recently Brian (1958) has suggested that the promotion of vegetative growth in *Kalanchoe* could result from the activity of a gibberellin-like substance. His suggestion applies equally well to the strawberry.

ACKNOWLEDGEMENTS

Much valued advice and help has been received on statistics from M. R. Sampford, and on the experimental work from P. A. Thompson.

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The Effect of PCIB on the Germination and Growth of Lettuce in the Presence of Thiourea

BY

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ABSTRACT

The joint action of the anti-auxin parachlorophenoxy isobutyric acid (PCIB) and thiourea on germination and growth of lettuce seed was investigated. PCIB alone did not affect germination or hypocotyl growth of lettuce. Root growth was stimulated by PCIB. In germination PCIB showed interaction at all the thiourea concentrations. All thiourea concentrations reversed the stimulation of root growth caused by PCIB, except the lowest thiourea concentration with the highest PCIB one.

In hypocotyl growth the two substances showed no interaction. These results are discussed in relation to the auxin and anti-auxin effects in germination and growth.

IN previous papers the joint action of thiourea with various compounds on germination and growth of lettuce was described (Poljakoff-Mayber, Mayer, Zacks, 1958*a*, 1958*b*; Mayer, Poljakoff-Mayber, and Zacks, 1958*a*, 1958*b*). Some of the compounds studied, e.g. ascorbic acid and diethyl thiourea, modified the effect of thiourea in these processes and interaction in their effect could be shown to occur. No such interaction was shown with IAA which did not modify the action of thiourea in germination and growth. It has, however, been shown (Poljakoff-Mayber, 1958; Poljakoff-Mayber, Mayer, and Zacks, 1958*b*) that IAA can affect the germination of lettuce seeds under certain conditions. In view of these results of the effect of IAA it was of interest to study the effect of an anti-auxin. The anti-auxin used was parachlorophenoxy isobutyric acid (PCIB) as the effect of this substance as an anti-auxin is generally accepted, whereas the function of other anti-auxins is still more than somewhat in doubt (Aberg, 1956, 1957). PCIB did not affect the germination and growth of those seeds (Poljakoff-Mayber, 1958) affected by IAA. It was therefore of interest to determine how PCIB affects seeds which do not react to IAA and whether it has any interaction with thiourea.

METHODS

Lettuce seed (variety Grand Rapids) which germinated to about 30 per cent. in water in the dark was used throughout the experiments. The seeds were germinated in the solutions tested at 26° C. in the dark. Germination and growth were determined as previously described (Poljakoff-Mayber, Mayer, and Zacks, 1958*b*). Four levels of PCIB 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} M. were used in the presence of three levels of thiourea, 1, 2·5, and 5×10^{-2} M.

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Results and Discussion

Lettuce seeds were germinated in combinations of various concentrations of PCIB and thiourea and in each of them alone the resultant percentage of germination and the mean root and hypocotyl lengths are recorded in Table 1. As will be seen, this table does not permit an easy interpretation of the results. The results in Table 1 were therefore subjected to statistical analysis, either after a transformation to Normit or Probit in the case of germination or directly in the case of root and hypocotyl length. The statistical method used was that of fitting regression lines either of the type $y = a+bx$ previously described (Mayer, Poljakoff-Mayber, and Zacks, 1958 *a* and *b*) or of the type

$$y = a+b_1x+b_2x^2,$$

according to Berkson 1955. In the first case the assumption was made that the data should fall on a straight line while in the second case it was assumed that optima or minima might occur in the regression line.

Table 1

The Effect of PCIB and Thiourea on the Germination and Growth of Lettuce

	Thiourea conc. $\times 10^{-2}$ M	PCIB concentration, M				
		0	10^{-7}	10^{-6}	10^{-5}	10^{-4}
Per cent. germination	0	24·9	24·9	28·8	23·2	29·9
	1·0	51·9	46·3	48·3	54·6	39·7
	2·5	61·9	41·6	49·5	58·4	45·7
	5·0	42·7	37·1	30·3	43·5	50·0
Mean root length, mm.	0	9·5	9·5	11·5	10·4	11·8
	1·0	7·1	8·2	8·5	7·6	11·3
	2·5	2·4	3·2	4·1	2·8	2·89
	5·0	1·3	1·3	1·4	1·3	1·3
Mean hypocotyl length, mm.	0	5·1	5·2	6·1	5·4	5·7
	1·0	3·6	4·3	4·9	3·55	4·5
	2·5	2·5	2·4	2·0	2·4	2·4
	5·0	1·3	1·2	1·1	1·25	1·2

Both types of analysis clearly showed that interaction exists between PCIB and thiourea in their effect on germination and root growth. Such interaction was entirely absent in the case of hypocotyl growth. The type and extent of the interaction varied with both the PCIB and thiourea concentrations.

The two statistical models used show different forms of the regression lines of the observed variable against the logarithmic concentration of PCIB. As biologically it is not possible to select one of the statistical models as the correct one, the regression lines are not presented or analysed here. The general trend of the results is as follows:

The addition of PCIB reduces germination as compared to thiourea alone in almost all cases (Table 1), despite the fact that PCIB alone does not affect germination. At the highest PCIB concentration (10^{-4} M.), the germination percentage rises with increasing thiourea concentration, the optimal curve typical for this batch of seeds with thiourea alone (Poljakoff-Mayber, Mayer, and Zacks, 1958) disappears. Root length increases with increasing PCIB concentration when applied alone as has also been shown by Burstrom (1951) and Almestrand (1957). When the two are applied together the increasing thiourea concentration always decreases root growth but this decrease is modified to different extents by the various PCIB concentrations. PCIB did not affect hypocotyl growth in any way. Increasing thiourea concentrations caused increasing depression of hypocotyl growth, both in the absence and presence of PCIB.

The existence of interaction between thiourea and PCIB in germination cannot be readily assigned to the anti-auxin-like properties of the PCIB. There is no evidence that IAA plays an essential part in germination, although it has been shown (Poljakoff-Mayber, 1958; Poljakoff-Mayber, Mayer, and Zacks, 1958*b*) that the germination of *deeply* dormant lettuce seed is stimulated by exogenous IAA. The root growth of such seeds is not affected by PCIB. In *less* dormant seeds IAA no longer stimulates germination, while PCIB promotes root growth. This indicates suboptimal auxin concentration in the former and optimal or supra-optimal auxin concentrations in the latter. If thiourea changes the auxin level of the seeds, the interaction of PCIB and thiourea could be explained. Such a change in auxin level is suggested by the findings of Blumenthal-Goldschmidt, 1958, who showed that lettuce seeds germinated in thiourea contain a neutral growth promoting substance which is absent in seeds germinated in water.

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'Nuclear Endosperm' or 'Non-cellular Endosperm'?

BY

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THE term 'nuclear endosperm' has established itself in botanical literature by frequent usage. It is applied to that type of endosperm in which a number of nuclei, derived by free nuclear division of the primary endosperm nucleus, lie in a common mass or layer of cytoplasm. Although this meaning of the term 'nuclear endosperm' is clear to all because it is defined and used in that sense for many years, yet it is an inappropriate one. Strictly speaking, it means endosperm composed of nuclear material alone and ignores the cytoplasm. This interpretation is neither far-fetched nor twisted if one compares the terms 'nuclear division', 'nuclear material', &c. In all these cases, as well as by dictionary meaning, the usage of the adjective 'nuclear' excludes cytoplasm, as for example, 'nuclear division' is distinct from cytoplasmic division (cytokinesis).

The term 'non-cellular endosperm' is a better one to denote what is ordinarily called 'nuclear endosperm' and was used by the present writer in 1938 (Ann. Bot., N.S., **2**, 535–6). V. H. Blackman, then editor of the Annals of Botany, agreed that the term 'nuclear endosperm' was unfortunate and that the term 'non-cellular endosperm' should be substituted for it. Since then Johanssen (1940) has used the term 'coenocytic endosperm' for 'nuclear endosperm' and, to a certain extent this also is correct, but it does not differentiate between the completely non-cellular condition and those states where there is wall formation in the endosperm but with some of the endosperm cells containing two or more nuclei. It is therefore suggested again that the term 'non-cellular endosperm' is a more appropriate one than the others.

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